„CAROL DAVILA", UNIVERSITY OF MEDICINE AND PHARMACY, BUCHAREST

DOCTORAL SCHOOL

MEDICINE

Impact of oral citrate treatment on residual renal microlithiasis following flexible digital ureteroscopy and Holmium laser lithotripsy

Doctoral supervisor:

PROF. UNIV. DR. GEAVLETE PETRIȘOR AURELIAN

Doctoral student:

ENE MIHAI ANDREI

Table of contents

List of published scientific articles

Articles published in specialized journals indexed ISI:

1. Mihai Andrei Ene, Petrișor Aurelian Geavlete, Cătălin Andrei Bulai, Cosmin Victor Ene, Bogdan Florin Geavlete. COMPARISON OF STONE-FREE RATE WITH CITRATURIA LEVELS IN KIDNEY STONES TREATED WITH DIGITAL FLEXIBLE URETEROSCOPY WITH HOLMIUM LASER LITHOTRIPSY. FARMACIA, 2022, Vol. 70, 6, 1168-1173. doi:10.31925/farmacia.2022.6.22. https://farmaciajournal.com/wp-content/uploads/art-22-Ene_Geavlete_1168-1173.pdf Indexare: Science Citation Index Expanded, SciVerse Scopus, Chemical Abstracts Service, EMBASE, SCImago Journal&Country Rank, IPA, British Library, SSCI, A&HCI, CPCI-S, CPCI-SSH, BKCI-S, BKCI-SSH, ESCI, CCR-EXPANDED, IC. Factor de impact = 1.6.

2. Mihai Andrei Ene, Viorel Jinga, Petrișor Aurelian Geavlete, Cosmin Victor Ene, Cătălin Andrei Bulai, Crenguța Sorina Șerboiu, Bogdan Florin Geavlete. STONE FREE-RATE EXPERIENCE IN POST-INTERVENTIONAL PATIENTS UNDERGOING CITRATES AND PYRIDOXINE ADMINISTRATION. FARMACIA, 2023, Vol. 71, 3, 573-580. doi:10.31925/farmacia.2023.3.16. https://farmaciajournal.com/wp-content/uploads/art-16-Ene_Geavlete_573-580.pdf Indexare: Science Citation Index Expanded, SciVerse Scopus, Chemical Abstracts Service, EMBASE, SCImago Journal&Country Rank, IPA, British Library, SSCI, A&HCI, CPCI-S, CPCI-SSH, BKCI-S, BKCI-SSH, ESCI, CCR-EXPANDED, IC. Factor de impact = 1.6.

Articles published in specialized journals indexed BDI:

1. Mihai Andrei Ene, Petrișor Aurelian Geavlete, Cătălina Elena Simeanu, Cătălin Andrei Bulai, Cosmin Victor Ene, Bogdan Florin Geavlete. The effectiveness of citrates and pyridoxine in the treatment of kidney stones. Journal of Medicine and Life. 2023 Iun, Issue 6. doi: 10.25122/jml-2023-0234. PMID: 37675156; PMCID: 10478649. https://medandlife.org/wp-content/uploads/9.-JML-2023-0234.pdf Indexare: Scopus, PubMed, ProQuest, Index Copernicus International, EBSCO, CNCSIS. Factor de impact = 0.385

Introduction

Renal lithiasis occurs in a proportion of 5-15% in the global population, not having a well-defined pattern, but especially more frequently among the male population, the percentage being in an increasing trend in the last 25 years [1]. As a potential risk factor for the development of chronic renal failure [2, 3], the problem of the existence of kidney stones has gained momentum in the urological world with the development of the pharmaceutical and technological industries.

The tendency to form calcium oxalate is directly proportional to the urinary concentration of calcium, oxalate, urate and inversely proportional to the concentration of magnesium and citrate [1]. Hypocitraturia represents a 16-63% risk factor in the occurrence of calcium-containing kidney stones [4, 5]. Numerous studies have demonstrated the effectiveness of potassium citrate in the prevention of nephrolithiasis, being a strong inhibitor of the lithogenesis process [6], its large-scale use also having considerable economic advantages [7, 8].

Concomitant with industrial technological progress, endoscopic operative techniques have been in continuous development in the last 50 years. The increase in the percentage of favorable results accompanied by the reduction in the rate of complications highlights the need for the use of flexible ureteroscopy on a large scale [9-11].

Mulvaney and Beck were the first to introduce the utility of the laser into the urological field. [12]. The Urology Clinic of the "Saint John" Emergency Clinical Hospital in Bucharest is one of the pioneers of flexible ureteroscopy in Romania. As the type of laser used in these surgeries, the Ho:YAG (Holmium:YAG) laser was used with remarkable results.

Following the practice of flexible ureteroscopy and Holmium laser lithotripsy of the stones detected at the renal level, multiple lithiasis fragments are dispersed throughout the pyelo-calyceal system. The opportunity to carry out a work, in which the impact of the use of citrates in the treatment of outstanding renal microlithiasis as a result of the minimally invasive surgical approach – digital flexible ureteroscopy with Holmium laser lithotripsy, can be studied, gained a favorable perspective.

I. General part

1. Renal lithiasis – general concepts

The prevalence and incidence of kidney stones differ according to geographic area, sex, age, as well as the composition and location of the stones. The explanation for these differences was given in terms of race, diet and food factors. The many global socio-economic changes of the past 50 years have reverberated in prevalence and incidence. Epidemiological surveys have been constantly re-evaluated, showing a prevalence rate between 4% and 20% [13].

Changes in the environment, functional and pathological changes in the systems and organs of the human body, genetic diseases, renal malformations, drugs, the presence of pathological processes at the renal level are risk factors preceding the appearance of renal lithiasis [14].

Depending on the etiology, lithiasis can have an infectious origin (ammonia-magnesium phosphate, carbon apatite, ammonium urate) or non-infectious (calcium oxalate, calcium phosphate, uric acid) secondary to genetic defects (cystine, xanthine, 2, 8 – dihydroxyadenine) [15] or drug administration.

An initial evaluation of any patient begins with a thorough medical history (especially in patients with a known history of urolithiasis) and a clinical examination. The dominant symptomatology in the pathology of renal lithiasis is pain (lumbar, radiated to the abdominal or external genital organs), variably accompanied by hematuria, fever, nausea, vomiting, fever, increased blood pressure, digestive manifestations [16].

Among the imaging studies used, we note the simple renovesical radiography as the first method of radiological exploration within the investigation protocol of a suspected lithiasis of the urinary tract. Abdominal ultrasound is usually a method for exploring the urinary tract because it is non-invasive (safe), inexpensive and widely available. Computed tomography without contrast material tends to be the gold standard in the diagnosis of urolithiasis in developed countries.

In the case of patients who present this pathology of urinary lithiasis for the first time, biochemical analysis of the calculus by X-ray diffraction or infrared spectroscopy is recommended. Regardless of the specific individual risk, all patients with kidney stone pathology should follow certain measures to prevent recurrences. A balanced diet and

lifestyle changes are the main goal in this direction. Some patients require specific prophylaxis which is established after the analysis of calculi and biohumoral samples. Pharmacological treatment is usually recommended to prevent recurrences.

Alkaline citrates can be used in the pathology of renal lithiasis of calcium oxalate, uric acid and cystine. As a mode of action, it contributes to the alkalinization of urine, corrects hypocitraturia and inhibits the formation of calcium oxalate crystals. As a dosage for children, 0.1-0.5 g/kg body weight/day is recommended, and for adults 5-12 g/day (14-36 mmol/day). This variation is due to the fact that the daily dose is adjusted according to the urinary PH [17-21].

.

2. Citrates - generalities

The energy status of the cells is evidenced by the intracellular level of citrate. It can thus be said that he acts like a moderator. When the energy needs are low, but the cellular level of ATP is greatly increased, the excess can be transported from the mitochondrial level to the cytosol, which can be achieved with the help of a mitochondrial citrate carrier [22]. The utility is found for the support of tissue functions of specialized cells [23] or for the biosynthesis of cellular lipids with increased proliferation rate [24].

Depending on the physiological needs of each individual, there is a balance between the availability of citrates and their excretion from the body. Citrate homeostasis is normally influenced by four major domains that frequently intersect and cooperate. These are represented by nutritional intake, renal clearance, cellular metabolism and bone remodeling.

Circulating citrates in the form of citrate salt from the blood are freely filtered at the level of the renal glomeruli. After glomerular filtration, reabsorption takes place in the proximal convoluted tubule. In this process, the amount of citrate eliminated at the tubular level never exceeds the resorptive capacity of citrate. The proximal convoluted tubule reabsorbs approximately 75% of the filtered citrate amount, thus becoming the main component of the final urinary citrate [25, 26].

The excretion of citrates/24 hours is considered to be the point of reference in highlighting possible alterations in their homeostasis [27]. Urinary citrate values range from 320 to 1260 mg/24 h, with a higher mean in women (approximately 680 mg/24 h) than in men (approximately 550 mg/24 h) [28, 29].

Considering the lower limit of normal citrate 320mg/day, we can talk about a severe citrate, when we encounter values of less than 100mg/day and a moderate-severe citrate, when the values are between 100 and 320mg/day. In any case, a question mark should be raised when excretion is low (below 640mg/day in men for example), constant monitoring should be rigorously applied [29].

Decreased renal function secondary to various risk factors or idiopathically induces hypocitraturia through two mechanisms, namely metabolic acidosis and decreased citrate excretion. In the case of distal renal tubular acidosis, two forms are distinguished: the complete form, characterized by hyperchloremic metabolic acidosis, hypokalemia and

increased urinary pH, and the incomplete form, which is manifested by normal values of serum electrolytes and inability to acidify urine following chloride loading ammonium [30]. In chronic renal failure there is a decrease in the glomerular filtration rate resulting in a decrease in the amount of filtered citrate. Excessive hypocitraturia is common in the final stages of this pathology [31].

In cases of hypocitraturic renal lithiasis, spontaneous crystallization and nucleation of calcium oxalate and calcium phosphate occurs. Potassium citrate is useful in that it has the role of inhibiting this process [32]. Metabolism of absorbed potassium citrate produces an alkaline load, increases urinary pH and also urinary citrate concentration as a result of increased citrate clearance. In relation to urinary pH, its optimal value in terms of this phenomenon, as well as the dissolution of already formed lithiasis complexes, is 6.5-7. In a study of patients with renal lithiasis, hypocitraturia and low urinary pH classified according to body mass index, Astroza et al. demonstrated that patients with high values of this parameter had smaller increases in urinary citrate and pH after initiation of potassium citrate treatment and needed frequent dose adjustment [33].

As for residual lithiasis fragments resulting from retrograde flexible ureteroscopy with Holmium laser lithotripsy, they can be extracted with the aid of the Dormia basket probe, and those that cannot be contained between its coils will be followed for spontaneous elimination.

Certain clinical studies, starting from this premise, have revealed a significant number of remaining microcalculi, generally of small size, thus creating a relative picture regarding the real "stone-free" status of the patients. Small calculus fragments migrate into the calyceal groups, mainly at the level of the lower calyceal groups, and then expect their spontaneous elimination.

As residual lithic fragments have been proven to constitute crystallization nuclei for future calculations, the chance of their reappearance starting from a residual fragment being between 21 and 59% during the next 25 years of evolution according to a study by Skolarikos et al . [34], the stake of obtaining a "stone-free" status becomes a benchmark for these cases.

II. Original contributions

3. Work hypothesis and general objectives

The concern for identifying the most effective and at the same time easy solutions for dissolving all remaining microcalculi at the level of the pyelo-calyceal tree after minimally invasive interventions has become more and more widespread globally. Following the review of the specialized literature, multiple references were recorded related to the role played by citrates in terms of the lytic treatment of uric acid stones, as well as regarding the substantial role of these compounds in the field of prophylaxis of the oxalo-crystallization phenomenon calcium and phosphate-calcium.

The aim of the study of the present paper is to analyze the effects of a combination of citrates on outstanding renal microlithiasis following digital flexible ureteroscopy and Holmium laser lithotripsy for renal stones.

Four main areas of interest were pursued:

1. A first essential aspect was the establishment of the proportion of cases that acquired the status of "stone-free" (absence of renal lithiasis);

2. Measurement with the help of imaging studies of the size of the remaining stones and comparison with the initial determination, carried out immediately after the endoscopic treatment;

3. The ability to expel the calculations in the 2 batches. This assumed that patients had recovered at least one stone during voiding.

4. Variation of urinary citrate and PH – the values of citrate/24h in the 2 arms were compared. It should be mentioned that the variation of citrate was studied between patients who achieved stone-free status and those who did not achieve this.

The available data were subjected to a thorough statistical analysis. It was aimed to establish the statistical significance of the differences that appeared in terms of achieving "stone-free" status, reducing the average size of residual stones, improving the ability to expel stones and the effect of the use of citrate and pyridoxine treatment on hypocitraturia and urinary PH .

4. General research methodology

4.1. Type of study and study population

A prospective, randomized study was conducted in which 198 patients were enrolled who received flexible ureteroscopy with Holmium laser lithotripsy for kidney stones between 1.2 and 2 cm in size, with residual kidney stones up to 6 mm in size, between January 2018 and June 2022 at the Emergency Clinical Hospital "Saint John" Bucharest. Among them, there were 101 men (51.01%) and 97 women (48.99%) with an average age of 48.8 years (between 20 and 84 years).

From the point of view of the body mass index (BMI) there were no patients who fell into obesity grade 2 or obesity grade 3. For a BMI <18.5 the percentage of patients was 27.78%. The highest percentage of patients was found in the BMI category between 18.5 and < 25, respectively 49.49%. 15.15% for BMI between 25 and < 30 and 7.58% for BMI between 30 and < 35 were the values for these latter categories.

The following day, a radiologist performed an ultrasonography for each individual patient to evaluate the remaining fragments (their number, location and size). As for the location of the residual stones, they were divided into two categories as follows: the first category was represented by the upper calyx and the middle calyx (30.3%), and the second by the lower calyx (69.7%).

After surgery, crystallographic examination by infrared spectroscopy or X-ray diffraction for extracted stones was a standard procedure. For inclusion in the study, their results had to report calcium oxalate, calcium phosphate, uric acid (13.64%) or a mixed composition of these (14.14%). Thus, 3 categories of stone types were obtained, considering the fact that those stones that had calcium in their composition (calcium oxalate - 41.92%, calcium phosphate - 11.11% or a combination of these two - 19.19%) were considered as unique category (72.22%). As a distribution within this last category, stones formed predominantly of calcium oxalate (58.04%), followed then by the combination of phosphate and oxalate (26.57%) and finally by those formed only by calcium phosphate (15.38%).

The patients were divided into two arms, the first with a number of 101 patients and the second with 97. The first group (Group A) benefited from a hygienic-dietary regime (the recommendation of a low-protein diet and a water intake substantial amount of

approximately 400 ml of water every 4 hours) along with treatment with a combination of potassium citrate, magnesium citrate and pyridoxine, and the second (Group B) only with a hygienic-dietary regime in accordance with the result obtained after cystal analysis and the same nutritional recommendations, without citrate combination treatment.

The amounts of compounds in each dose of the combination mentioned above were 2703 mg of potassium citrate (975 mg of potassium), 376 mg of magnesium citrate (60 mg of magnesium) and 25 mg of pyridoxine per sachet. This combination is characterized by a significant intake of citrates (respectively of 25 mEq potassium citrate and 5 mEq magnesium citrate per dose) and pyridoxine. The frequency of compound administration was twice a day at twelve-hour intervals (fixed time interval).

As inclusion criteria in the study, as mentioned above, the patients, in addition to oxalate and/or calcium phosphate stones, uric acid or a combination of these objectified by crystallographic analysis, signed an informed consent. Also, they did not have urinary infections, endocrinological pathologies, neoplastic pathologies or cytostatic treatment in the antecedents or in progress.

Patients with allergies to previous citrate treatments, those with pathology associated with a consecutive contraindication to citrate administration (severe liver or kidney failure) and pregnant patients diagnosed with lithiasis during pregnancy were not included in the study. In the case of another type of lithiasis than those mentioned above, patients were also excluded.

Cases with major comorbidities (such as advanced heart or respiratory failure, severe coagulation disorders, stroke or recent myocardial infarction) constituted an impediment to study enrollment.

The urine analysis was mainly characterized by the assessment of urinary citrate and pH, initially preoperatively, then at 3, 6, 9, and 12 months, respectively.

The decision to include in the study and to continue the subsequent monitoring of each patient was given by a maximum size of outstanding fragments of 6 mm. Ultrasonography was used as an imaging modality for evaluation at 3, 6, 9 and 12 months, respectively. In cases where calculi were identified following this examination, computer tomography was used to determine their exact number, location, and size.

Evaluations at intervals of 3, 6, 9 and 12 months were aimed at tracking some parameters regarding the composition of the urine, respectively the dimensions of the outstanding renal lithiasis. The main directions of interest were represented by:

1. Acquiring "stone-free" status (absence of renal lithiasis);

2. Reducing the average size of residual calculations;

3. The ability to expel the calculations in the 2 batches;

4. Variation of urinary citrate and PH.

4.2. Apparatus and equipment

The equipment used to perform digital flexible ureteroscopy surgery with Holmium laser lithotripsy was represented by:

• flexible ureteroscope 8.4 Fr URF-V3 2001982 (Olympus, Hamburg, Germany);

• VISERA ELITE II OTV-S200 video system with All-in-One 2D processor and light source;

• MediCap USB-300 HD recording system;

• LMD-X310S/LMD-X550S monitor – 31" LCD;

• sterile water with irrigation liquid;

• 10/12 Fr. ureteral access sheath;

• probe with basket type Dormia 2.2 Fr (Colopast, Humlebaek, Denmark);

• Dornier SingleFlex laser fiber 270 microns;

• Holmium Dornier Medilas H Solvo 35 laser;

• stainless steel guide (Teleflex Medical, Kernen, Germany).

4.3. Intervention stages

Any surgical intervention is preceded by preliminary measures such as the pre-operative preparation of the patients, the instruments necessary for the intervention, anesthesia and the positioning of the patients on the operating table. It is important to mention the care of the medical staff to respect the optimal asepsis conditions.

From the point of view of the positioning of the patients, they need to be located in the standard lithotomy position, with the lower limb contralateral to the approached kidney located slightly downward compared to it and in a hyper-abduction position [35]. This position allows the operator increased mobility during the surgical intervention, the contralateral lower limb thus not representing an impediment regarding the maneuverability of the working tools.

All surgical interventions, given the strict inclusion criteria in the study, were performed under spinal anesthesia.

The first stage is represented by the introduction of the urethrocystoscope at the level of the urinary bladder. The urethra and bladder are inspected for possible associated pathology, then attention is directed to the ureteral opening ipsilateral to the renal pathology.

The safety guide is inserted at the level of the ureteral orifice which ascends under radiological control to the level of the pyelocalyceal system. Its caliber is reduced - ureteral dilatation is performed so that the ureteral access sheath can be easily inserted. The dilator has a Teflon-like composition and is wrapped by a coaxial sheath. It will be ascended on the guide until its distal end reaches pelvic level. This can be verified by introducing contrast material under radiological control. This dilator is extracted while the sheath remains in place. Simultaneously with its extraction, the guide is also extracted.

The next stage is represented by the introduction of the flexible digital ureteroscope under optical control through the ureteral access sheath up to the level of the pyelocaliceal system where the kidney stones are identified. The Holmium laser fiber is then inserted to at least 2 mm from the distal end of the ureteroscope, so that there is no damage to the working channel or damage to the optical system. The fiber needs to be located near the calculation, possibly having contact at this level, before activation. The desired mode of lithotripsy is then selected and the existing lithiasis is addressed.

After lithotripsy with the aid of the Dormia basket probe, lithic fragments are recovered which will later be sent for crystallographic analysis. Both the ureteroscope and the ureteral access sheath are then extracted under optical control so that the ureter can be inspected. The last step is the installation of a urethro-vesical Foley catheter, which is removed 12-24 hours postoperatively.

Considering the objectives of the study, among which the ability to expel the stones is highlighted, it was decided not to mount JJ probes at the end of the interventions.

4.4. Statistical analysis

The data obtained were coded and later entered into a database in the SPSS v. 20.0 program (SPSS Inc., 2012) with the help of which they were statistically analyzed.

In the descriptive statistical analysis, the mean, standard deviation, median, minimum and maximum of the distribution were determined for continuous variables, while absolute and relative frequencies were determined for categorical variables. The main endpoint was represented by the "stone-free" rate at established time intervals after the endourological treatment. The chi-square ($\chi 2$) test was used to investigate this endpoint for two independent samples.

The secondary endpoint was the ability to expel in both groups after the established interval of conservative treatment. The expulsion rate was synonymous with the fact that each patient recovered a stone following voiding. It is important to note that this did not mean achieving stone-free status by default, since there were patients who had multiple stones and successfully expelled one or more, but failed to achieve this status . The chi-square test ($\chi 2$) was also used to investigate this endpoint, body mass index, location of residual renal lithiasis, and stone composition in each group. Another endpoint studied was the size of the residual stones at established time intervals from the start of the endourological treatment. For this objective, the Wilcoxon Rank Sum test was used for each group separately, as well as the bifactor ANOVA (analysis of variance) test, to evaluate the differences between the groups at the start time, i.e. immediately postoperatively and the effects after treatment, i.e. at the interval of set time (3,6,9 and 12 months) from its start. If the distribution of outcome variables was too skewed to the right, variables were logarithmically transformed based on the natural logarithm. The significance level was 0.05, so p values below 0.05 obtained in the inferential tests were considered statistically significant. The two-factor ANOVA test was also used to assess the variation in urinary PH.

Regarding the last field of interest studied, namely the variation of the citrate, for the inferential analysis the methodology was the following: the variables of interest were the values of the citrate/24h at the time of admission to the hospital before the start of the treatment and considering that these variables represent concentrations (titers), their

central tendency was evaluated using the geometric mean and not the arithmetic mean; thus, two-way inferential Paired t-tests (which compared the values of citrate/24h at baseline and at 3,6,9 and 12 months) and two-way Welch t-tests (which compared the values of citrate in patients with a favorable endpoint with the value of in patients with an unfavorable endpoint) were made on the natural logarithm values (based on e) of the citraturias at 24h, the testing of statistically significant differences between the geometric means being equivalent to the testing of statistical significance between the natural logarithms of the arithmetic means. All tests had a significance level of $\alpha = 0.05$, being considered statistically significant for a value of $p < 0.05$.

5. Results

5.1. Results obtained postoperatively – batch parameters

The 198 patients were assigned to 2 arms. The first arm had a number of 101 patients and followed a hygienic-dietary regimen established by crystallographic analysis accompanied by a combination of citrates and pyridoxine, while the 2nd arm had a number of 97 patients, following only hygienic-dietary regimen.

A descriptive statistical analysis of the variables used in the study was performed.

Table V.1. Demographic data

	Group A (N = 101)	**Group B (N = 97)**	**P -value**
Age			**0.539**
Mean (SD)	**49.44 (14.7)**	**48.13 (14.9)**	
Median [Min, Max]	**48.0 [21.0, 84.0]**	**47.0 [20.0, 83.0]**	
Sex			**0.481**
F	**47 (46.53%)**	**50 (51.55%)**	
M	**54 (53.47%)**	**47 (48.45%)**	
BMI			**0.768**
<18.5	**25 (24.75%)**	**30 (30.93%)**	
18.5 to <25	**51 (50.5%)**	**47 (48.45%)**	
25 to <30	**17 (16.83%)**	**13 (13.4%)**	
30 to <35	**8 (7.92%)**	**7 (7.22%)**	

(Class 1)			
Residual stone position			**0.619**
Superior and Middle Calix	**72 (71.29%)**	**66 (68.04%)**	
Inferior Calix	**29 (28.71%)**	**31 (31.96%)**	
Stone composition			**0.237**
Uric acid	**15 (14.85%)**	**12 (12.37%)**	
Calcium Oxalate/Phosphate	**68 (67.33%)**	**75 (77.32%)**	
*Calcium Oxalate	**39 (38.61%)**	**44 (45.36%)**	**0.932**
*Calcium Phosphate	**10 (9.9%)**	**12 (12.37%)**	
*Calcium Oxalate and Phosphate	**19 (18.81%)**	**19 (19.59%)**	
Mixt	**18 (17.82%)**	**10 (10.31%)**	

Regarding the gender distribution, in Group A a percentage of 53.47% of male patients was found, compared to a percentage of 46.53% of female patients. For Group B, a percentage of 51.55% female patients was identified, compared to 48.45% male patients. Thus, there were no statistically significant differences between the two groups, p value = 0.481. Similarities between the two groups could also be highlighted regarding age groups. The mean for group A was 49.44, while for group B it was 48.13. No statistical differences were identified between the two groups, p = 0.539.

From the point of view of body mass index (BMI) there were no patients who could be classified in Class 2 or Class 3 obesity. For BMI<18.5, there were 24.75% of patients for Group A and 30.93% of patients for Group B. For BMI between 18.5 and 25, there were 50.5% of patients for Group A and 48.45% for Group B. 16.83% of patients for Group A and 13.4 % patients for Group B were identified for BMI from 25 to <30. In the last category, with BMI from 30 to <35, patients were found for Group A 7.92% and

7.22% for Group B. At the $\chi 2$ test, $p = 0.768$. Thus it was demonstrated that there were no statistical differences between the two groups in terms of this studied parameter.

As for the location of residual calculi, they were divided into two categories as follows: the first category was represented by the upper calyx and the middle calyx, and the second by the lower calyx. 71.29% of patients in Group A fell into the first category and 28.71% into the second. For Group B, a prevalence of 68.04% patients was identified for the first mentioned category and 31.96% for the second. There were no statistically significant differences between the two groups, at the $\chi 2$ test, $p = 0.619$.

Following the postoperative crystallographic analysis, oxalate/calcium phosphate lithiasis was the most common in both groups (67.33% for Group A and 77.32% for Group B). For uric acid stones, a percentage of 14.85% was reported for Group A and 12.37% for Group B. In 10.31% of cases a mixed composition was found for Group B, compared to Group A, where the percentage was much higher , respectively 17.82%. At the $\chi 2$ test, $p = 0.237$, thus there were no statistically significant differences between the two groups.

The $\chi 2$ test was also used to analyze oxalate and/or calcium phosphate stones. For Group A a percentage of 38.61% was identified for calcium oxalate lithiasis, 9.9% for calcium phosphate lithiasis and 18.81% for oxalate and calcium phosphate lithiasis. For group B, these percentages were 45.36% for calcium oxalate lithiasis, 12.37% for calcium phosphate lithiasis, and 19.59% for oxalate and calcium phosphate lithiasis. No statistically significant differences were identified between the groups, p value = 0.932.

5.2. Results obtained after 3 months of treatment

After 3 months of treatment, a total stone-free rate of 76.26% was identified in the patient group. In Group A the stone-free rate was 83.17%, and in Group B it was 69.07%. At the $\chi 2$ test, $p = 0.02$, equivalent to the fact that the results obtained are statistically significant.

Evaluating the next endpoint of the study, the ability to expel stones at 3 months for Group A was 35.64%, and for Group B 22.68%. In the $\chi 2$ test, $p = 0.045$, which means that the results were statistically significant and revealed that patients treated with citrate combination had a better stone expulsion rate.

A statistical analysis of variables, baseline mean after digital flexible ureteroscopy with Holmium laser lithotripsy and mean after 3 months of treatment, was used to assess the last endpoint of the study. They were considered only for patients who after 3 months of treatment presented residual calculi. In Group A, a reduction in the average diameter of residual stones at 3 months of 2.35 mm was identified. At the Wilcoxon Rank Sum test, $p = 0.000$, thus concluding that the results obtained were statistically significant. In Group B, a reduction in the average diameter of residual stones at 3 months of 1.7mm was also identified. And these results were statistically significant by the Wilcoxon Rank Sum test, $p = 0.000$. Analyzing the 2 groups using the bifactorial ANOVA (analysis of variance) test, the p value = 0.035, thus revealing statistically significant results.

The pH variation in the citrate combination treatment group started from an initial mean value of 5.325, while for the diet only group the initial mean value was 5.316. At 3 months after the start of treatment, a mean pH value of 5.756 was determined for group A, while for group B it was 5.416. The bifactorial ANOVA (analysis of variance) test was also used to evaluate the PH variation within the two samples, the p value < 0.01 demonstrating statistically significant results.

For the results obtained in the last field of interest of the study, namely the variation of citraturia - the statistical analysis was studied between the patients who achieved the "stone-free" status (151 patients) and those who did not achieve this goal (47 patients) .

From the point of view of the citrate values, the geometric mean for the patients who achieved the "stone-free" status was 308.55 mg/dl at the beginning of the treatment, respectively 644.11 mg/dl after 3 months.

For patients in Group B, the geometric mean was 269.4 mg/dl at the beginning of treatment and 388.53 mg/dl after 3 months.

As these variables represent concentrations (titers), their central tendency being evaluated with the help of the geometric mean, the inferential Paired t two-way tests performed on the natural logarithmic values were used for the statistical analysis. Thus, for the patients where the "stone-free" status was obtained, the value $p < 0.01$ was highlighted, and for the patients in whom this status was not obtained, the value $p = 0.001$. Two-way Welch t tests were used on natural logarithmic values, p value = 0.001. Considering the

fact that all tests had a significance level of $\alpha = 0.05$, being considered statistically significant for a value of $p < 0.05$, the results obtained were statistically significant.

5.3. Results obtained after 3 months of treatment

A total stone-free rate of 79.8% was identified in the group of patients after 6 months of treatment. The stone-free rate was 86.14% for Group A, while for Group B it was 73.2%. The results obtained are statistically significant, with the $\chi 2$ test, $p = 0.023$.

The ability to expel stones at 6 months for Group A was 38.61%, and for Group B 24.74%. In the $\chi 2$ test, $p = 0.036$, which means that the results were statistically significant.

In Group A, a reduction in the average diameter of the residual stones was identified 6 months after the start of treatment of 2.23 mm, and in Group B of 1.61 mm. In the Wilcoxon Rank Sum test, $p = 0.001$ for Group A and $p = 0.000$ for Group B, which meant that the results obtained were statistically significant. Analyzing the 2 groups using the bifactorial ANOVA (analysis of variance) test, the p value was 0.018, so the results were statistically significant.

At 6 months after the start of treatment, the average value of PH for group A was 6.696, and for group B 5.611. Using the bifactorial ANOVA (analysis of variance) test to evaluate the PH variation within the two samples, a $p < 0.01$ value was obtained, thus demonstrating statistically significant results.

The variation of citraturia between patients who achieved stone-free status (158 patients) at 6 months and those who failed to achieve this goal (40 patients) is presented in the following paragraphs.

From the point of view of citrate values, the geometric mean for patients in Group A was 301.15 mg/dl at the beginning of treatment, respectively 733.65 mg/dl at 6 months.

In Group B the geometric mean was 289.56 mg/dl at the beginning of treatment and 469.73 mg/dl after 6 months.

In the inferential Paired t two-way tests performed on the natural logarithmic values, for the patients where the "stone-free" status was obtained, a value of $p < 0.01$ was found, and for the patients in whom this status was not obtained, the value of $p < 0.01$. In two-

tailed Welch t-tests on natural log values, p-value = 0.000. All results obtained were statistically significant.

5.4. Results obtained after 9 months of treatment

After 9 months of treatment, a total stone-free rate of 82.32% was identified. In Group A 89.11 % was the value for the stone-free rate, and in Group B 75.26 %. In the $\chi 2$ test, $p = 0.011$, which meant that the results obtained are statistically significant.

At 9 months, the ability to expel stones for Group A was 40.59%, and for Group B 25.77%. The results obtained were statistically significant, with the $\chi 2$ test, $p = 0.027$.

In Group A a reduction in the mean diameter of residual stones of 2.27 mm ($p = 0.002$ by the Wilcoxon Rank Sum test) and 1.54 mm in Group B ($p = 0.000$ by the Wilcoxon Rank Sum test) was identified at 9 months of treatment. In the bifactorial ANOVA test, the p value = 0.014, thus highlighting the fact that the results obtained were statistically significant.

For group A, an average value of PH of 6.759 and for group B of 5.636 was determined 9 months after the start of treatment. To evaluate the PH variation in the two groups, the bifactorial ANOVA test was used, the value $p < 0.01$ being similar to statistically significant results.

The statistical analysis for the variation of citraturia at 9 months was studied between the patients who obtained the "stone-free" status (163 patients) and those who did not acquire this status (35 patients).

From the point of view of the citrate values, the geometric mean for the patients in whom the "stone-free" status was obtained was 298.86 mg/dl at the beginning of the treatment, respectively 874.25 mg/dl at 9 months.

For patients in the other group, the geometric mean was 298.38 mg/dl at the beginning of treatment and 550.43 mg/dl after 9 months.

The value $p < 0.01$ was obtained in the two-way paired t inferential tests performed on the natural logarithmic values for both groups. The p value < 0.01 was highlighted by

the two-way Welch t test on the natural logarithmic values. It is thus noted that the results were significant from a statistical point of view.

5.5. Results obtained after 12 months of treatment

After 12 months of treatment, the total stone-free rate was 83.83%. The stone-free rate in Group A was 90.1% and 77.32% in Group B (Figure 5.1.). At the $\chi 2$ test, $p = 0.015$, consistent with the fact that the results obtained are statistically significant.

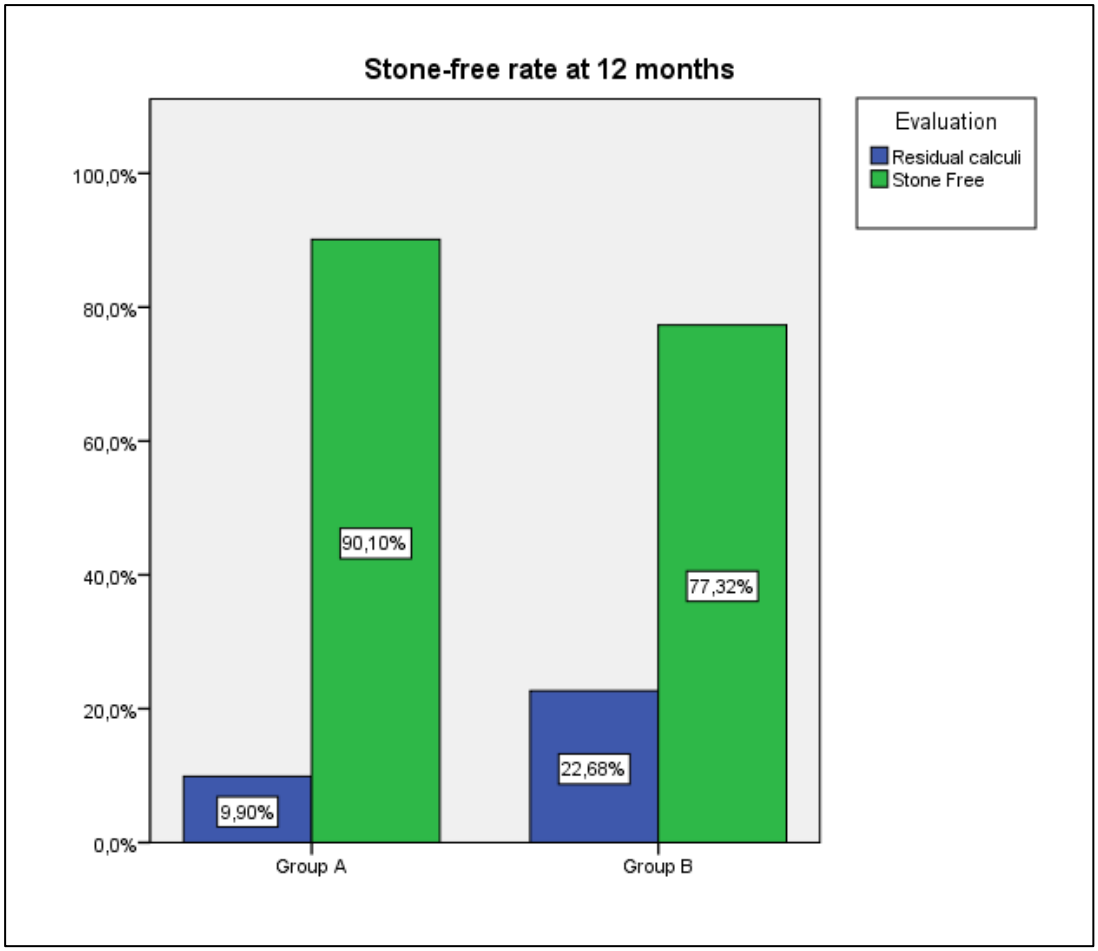

Figure 5.1. Stone-free rate at 12 months

Regarding the ability to expel stones at 12 months, a percentage of 41.58% for Group A and 25.77% for Group B was highlighted (Figure 5.2.). The results being statistically significant (by the $\chi 2$ test, $p = 0.019$), we can state that patients treated with a combination of citrates and pyridoxine had a better rate of stone expulsion.

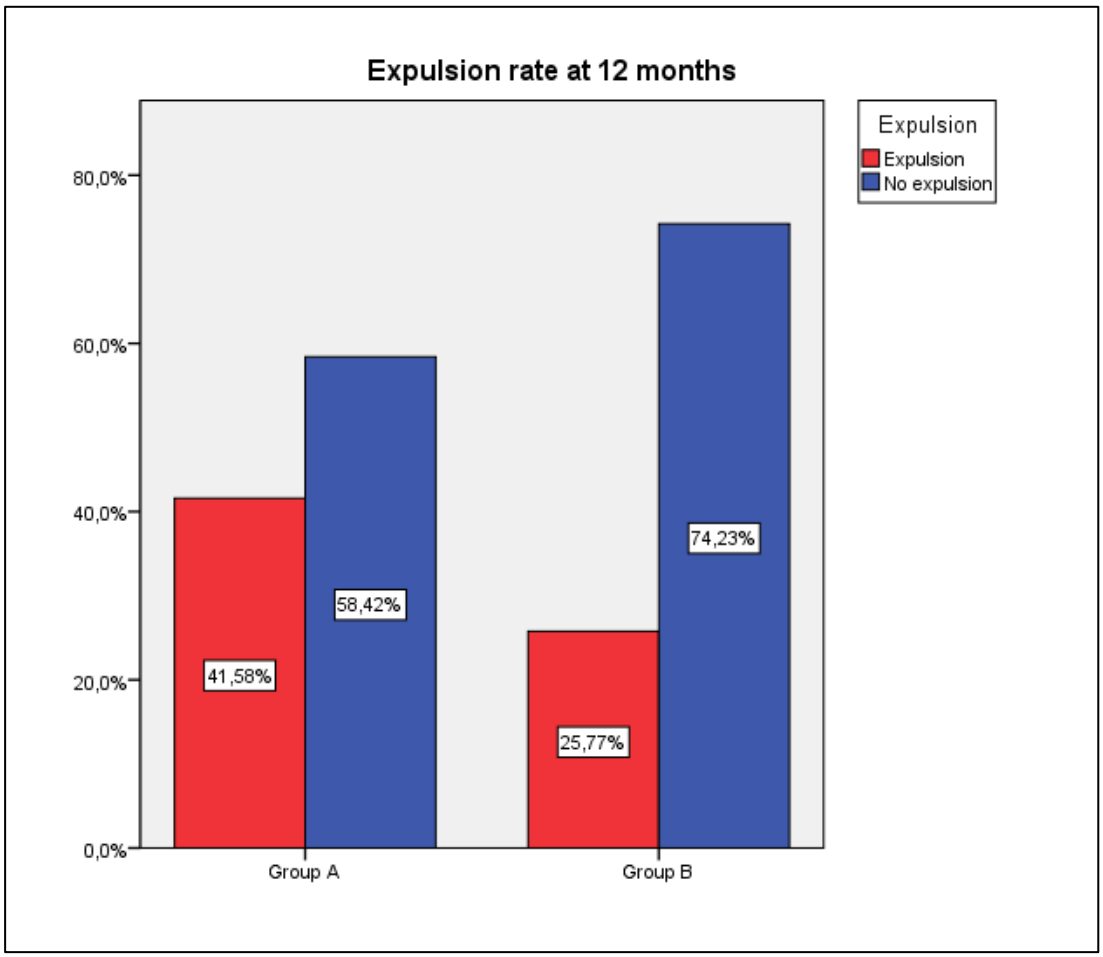

Figure 5.2. Expulsion capacity at 12 months

In Group A, a reduction in the mean diameter of residual stones at 12 months of 2.3 mm was found ($p = 0.001$ by Wilcoxon Rank Sum test). In Group B this value was 1.5mm ($p = 0.000$ with the Wilcoxon Rank Sum test). Analyzing the 2 groups using the bifactorial ANOVA test, $p = 0.012$, which meant that the results were statistically significant (Figure 5.3.). Levene's test indicated a value of 0.219 (greater than 0.05), thus it can be stated that there were homogeneous variances between the 2 groups. Following the obtained results, the essential role of citrate treatment for reducing the diameter of residual stones is identified ($F_{A,B} = 6.673$, $p < 0.05$).

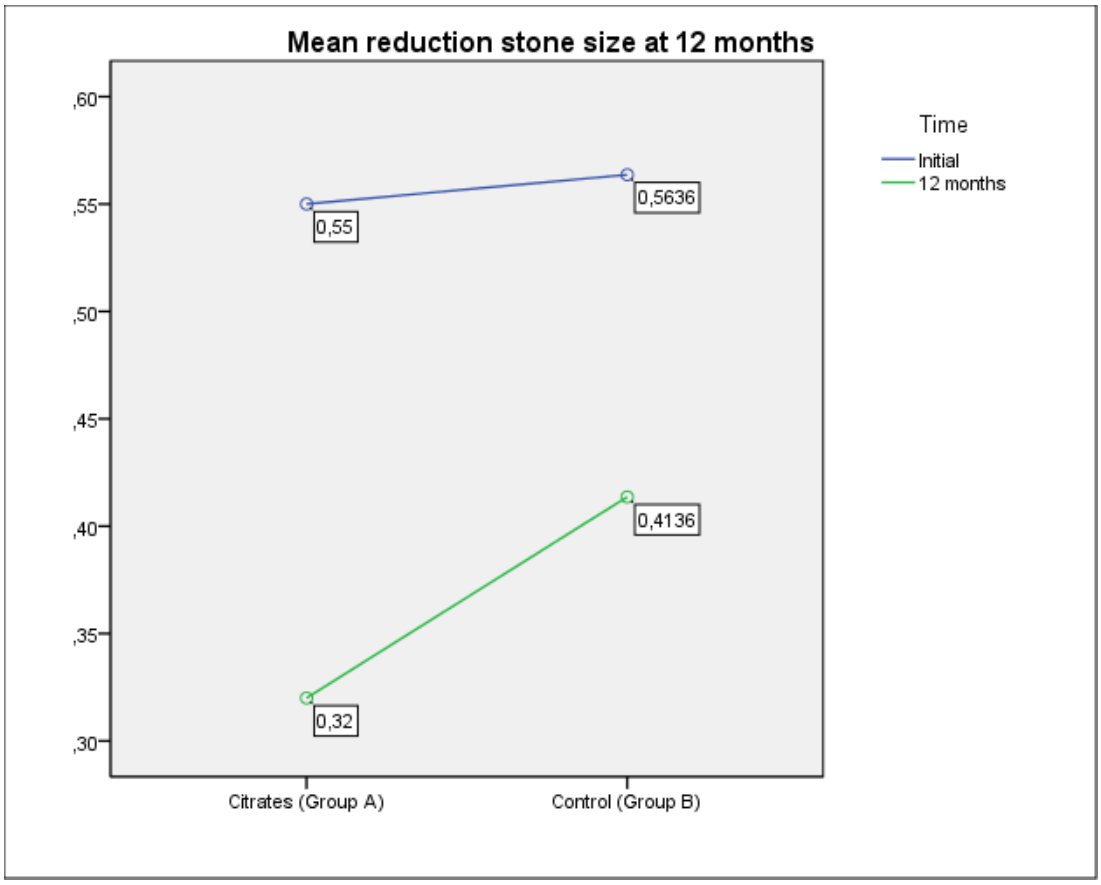

Figure 5.3. Mean reduction stone size at 12 months

The last change in pH was determined 12 months after the start of treatment. Thus, for group A an average PH value of 6.845 was obtained, while for group B it was 5.688. Using the bifactorial ANOVA test, the p value < 0.01 concluded statistically significant results (Figure 5.4.). There were homogeneous variances between the 2 groups, Levene's test indicating a value of 0.113 (> 0.05). Thus, following the obtained results, it can be stated that the treatment with a combination of citrates and pyridoxine has a beneficial effect in order to increase urinary PH ($F_{A,B} = 242{,}640$, $p < 0.05$).

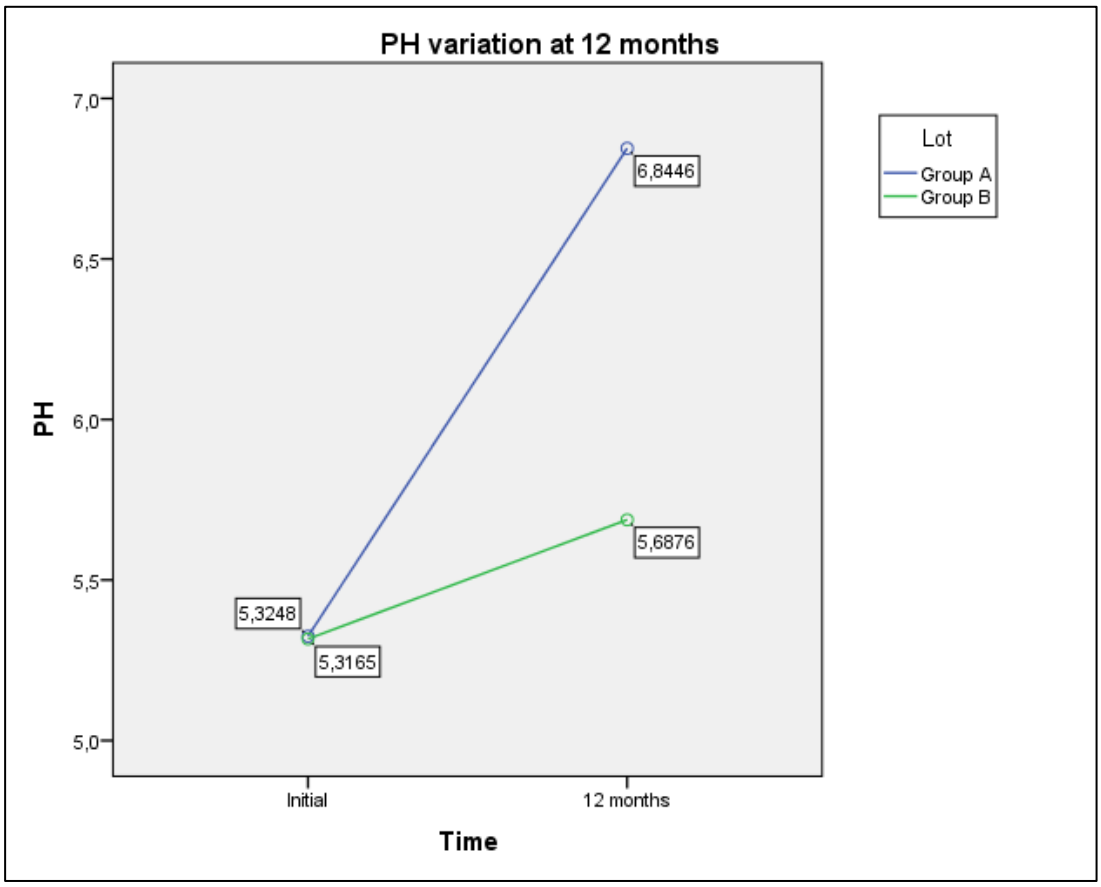

Figure 5.4. PH variation at 12 months

The variation of citraturia at 12 months was studied between the patients in whom the "stone-free" status was obtained (166 patients) and those with residual lithiasis (32 patients).

The geometric mean of the citrate values for the patients who achieved "stone-free" status was 301.21 mg/dl at the beginning of treatment, respectively 978.42 mg/dl at 12 months.

For patients in the residual lithiasis group the geometric mean was 286.4 mg/dl at the beginning of treatment and 621.1 mg/dl at 12 months.

As these variables represent concentrations (titers), their central tendency being evaluated with the help of the geometric mean, the inferential Paired t two-way tests performed on the natural logarithmic values were used for the statistical analysis, which revealed values < 0.01 both for the patients where it was obtained the "stone-free" status, as well as for those with residual lithiasis. Two-way Welch t tests were used (which compared the citrate values in patients where the "stone-free" status was obtained with the citrate value in patients with residual lithiasis) on the natural logarithmic values, obtaining

a value of $p < 0.01$ (Figure 5.5 .). We can thus affirm the fact that all the results obtained within this direction of research were significant from a statistical point of view. Following these data, the beneficial effect of the combination of potassium citrate, magnesium citrate and pyridoxine for increasing the level of citrate is observed.

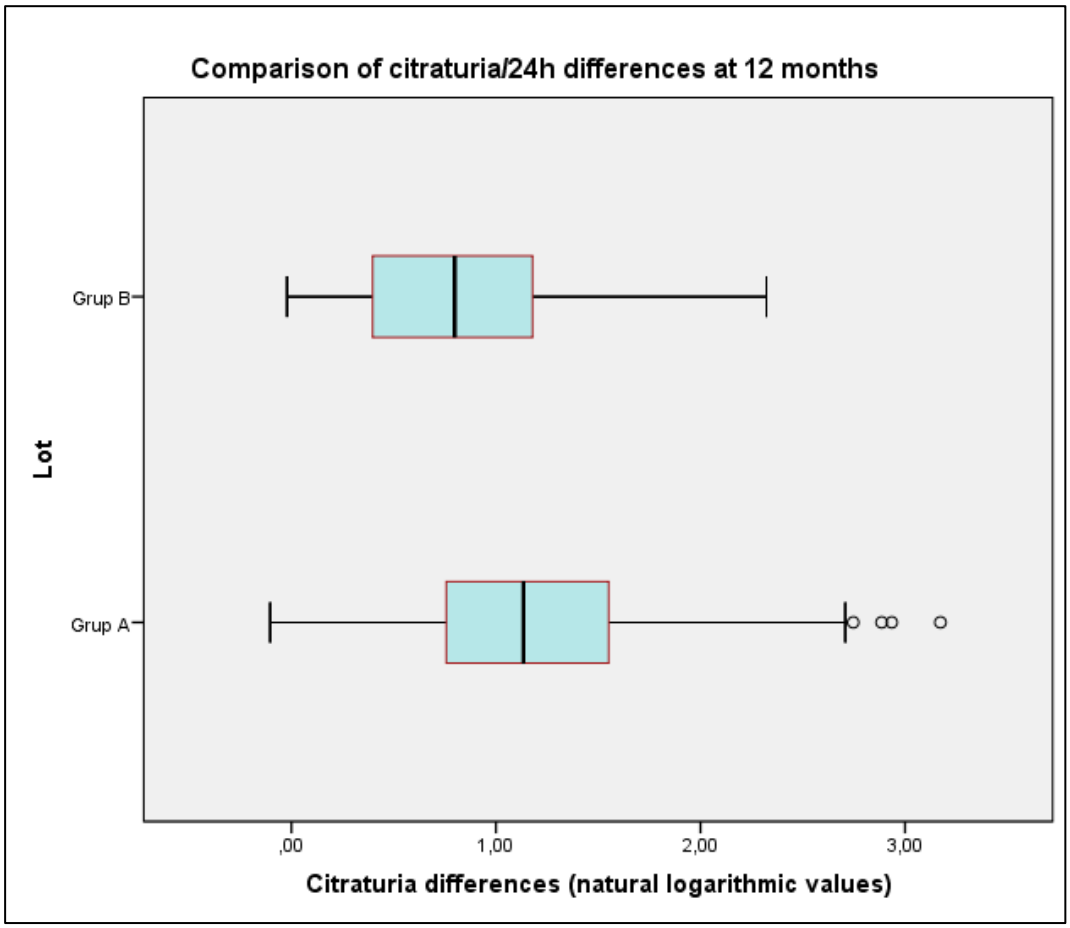

Figure 5.5. Comparison of citraturia differences between groups at 12 months of treatment

6. Discussions

Ferraro et al. recently performed a literature review that consisted of a search for a number of databases using keywords such as "renal lithiasis", "renal stones", "nephrolithiasis". The risk factor with the greatest impact was the lack of ingested fluids, an amount of 200 ml of fluids consumed daily reduced the risk of kidney stones by 13%. Citrates represent an important protective factor, their increased presence in urine being associated with a decrease in oxalo-calcium crystallization and by increasing urinary PH. Also, potassium citrate (found in citrus juices) has an important role in preventing the formation of renal lithiasis, this premise being the basis for administering potassium citrate to the patients in our study. Thus, if we refer to the evidence found by this review, we notice that also in the present study the level of urinary citrate is an extremely important protective factor for the occurrence of renal lithiasis [36].

A meta-analysis performed in 2015 on a cohort of 477 adult patients with predominantly calcium oxalate urolithiasis evaluated the impact of potassium citrate salts, combined sodium and potassium citrate salts, and combined potassium citrate salts. potassium and magnesium in the prevention and treatment of this pathology. Following the results obtained, an important rate of reduction in stone size was demonstrated in patients who received citrate salts and a reduced incidence of renal lithiasis recurrence [17].

One of the most common treatable causes of kidney stones is hypocitraturia. For the first time in the literature this was reported by Boothby and Adams in 1934 [37], later confirmed by Kissin and Locks in 1941 [38]. Vescini et al. is a study that found a very high prevalence of hypocitraturia in kidney stone patients, which is estimated to be between 8% and 68.30% [27].

With the help of electron microscopy, X-ray diffraction, Fourier transform infrared spectroscopy and the nanoparticle analyzer, it was highlighted that calcium phosphate, uric acid and ammonia-magnesium phosphate particles coexist in the lithiasis formed from calcium oxalate. As a conformation, almost all of them showed sharp corners with sizes varying from a few nanometers to a certain number of microns. The tendency to aggregation was identified, thus realizing the premises of the lithogenesis potential [39].

Starting from these notions, Chao-Yang Duan et al. conducted a study with a group of 13 patients with calcium oxalate lithiasis who were given potassium citrates, analyzing their potential to change the spatial configuration of the crystallites. The results showed a

decrease in crystallite size (from 524±320nm to 354±173nm), as well as an increase in urinary pH. Thus, the beneficial effect of using citrates in oxalic lithiasis was demonstrated [40].

The mentioned results support the fact that the use of the combination of citrates and pyridoxine is beneficial in the treatment of renal lithiasis with hypocitraturia, significantly improving this parameter, a fact also highlighted by Prezioso et al. where one of the conclusions is that despite costs and less favorable gastrointestinal side effects, the administration of alkaline citrate salts is recommended in the medical treatment of renal lithiasis with hypocitraturia [41].

The drug therapy used in our study considerably increased the citrate values, similar results were obtained in a study carried out by Koenig et al., on 14 healthy volunteers, where the variations of the citrate were compared in the actual administration of potassium citrate, respectively magnesium citrate with their combined administration [42].

The study by Rodgers et al. is a study done on 4 groups of patients, from which urine was collected before and after the administration of citrate, and then examined with the help of a specialized program (JESS) in order to determine the form in which different elements (Ca, Mg, etc) from its composition. The authors link the efficacy of citrate treatment to urinary pH, arguing that the favorable response to citrate therapy is not only dependent on the presence of a large amount of urinary citrate, but also on urinary pH. This may explain the different response of patients to citrate therapy, which was also observed in our study [43].

Another study with results similar to those obtained by Rodgers et al. is the one performed by Conte et al. in which a group of 119 patients who received potassium citrate treatment for 6 months was compared with a group of 16 patients who did not receive treatment. Patients who received treatment were previously divided into 2 groups, the first with 61 patients with hypocitraturia, and the second with 58 patients with other urinary pathologies with hypocitraturia or normocitraturia. As results, for both groups that benefited from the treatment, increases in citrate values were identified, especially for patients with hypocitraturia (from 198 +/- 13 to 476 +/- 35 mg/24 h) [44].

.

Using computed tomography, Coll et al. conducted a study which highlighted the fact that the chances of removal for a calculus under 5mm are 75% regardless of its location. Segura et al. noted that the chances of removal of a calculus located in the distal ureter smaller than 5 mm are between 71 and 98%, and for those of 5-10 mm with the same location, they are approximately 25-53% [45, 46] . These results formed a starting point on which we relied when composing the study design.

Pak et al. highlighted the fact that a low level of urinary citrate, which in some cases has been associated with other metabolic disorders such as hypercalciuria and hyperuricosuria, is associated with urinary lithiasis; also a therapeutic regimen with citrate 20mEq 3 times a day, over a period that was between 1 and 4.33 years, determined a 97.80% decrease in the possibility of new stone formation and remission was also achieved in 79.8% cases [47]. Although in our study other therapeutic regimens were used, as well as a shorter follow-up period, the results obtained support those of the mentioned study.

Spivacow et al. tracked the remission rate of renal lithiasis in a group of 35 patients treated with a mean dose of 45.40 ± 15.20 mEq/day of potassium citrate, and the result obtained was a remission rate of 91%, similar to that of our study (90.1% at 12 months of treatment) [48].

A controlled clinical study by Elbaset et al. on a sample of 150 patients randomized into 3 groups: the first group benefited from oral citrate treatment, the 2nd from ESWL and the last from combined therapy, respectively ESWL and citrate, aimed to determine the stone-free rate 3 months after initiation of therapy. The best results were obtained by patients from the 3rd category [49]. This aspect is similar to the results of our study.

According to Rosa et al. the use of various combinations of citrates is known to reduce the risk of kidney stone formation. Thus, citrate supplementation may have an important role in expulsion therapy after SWL [50]. These results are similar to those obtained in the present work, the combination of citrates used improving the expulsion capacity after 12 months of treatment, respectively 41.58%.

7. Conclusions and personal contributions

7.1. Conclusions

The therapeutic management of lithiasis recurrences requires both changes in lifestyle, diet and pharmacological interventions. The decisions that can lead to treatment must be based on the metabolic evaluation and each type of stone, as well as the associated comorbidities. Considering the fact that all stones are caused by urinary supersaturation with the material they are made of, the goal is to reduce or eliminate this condition.

Considering the increase in the prevalence of renal lithiasis in recent years, it is necessary to progress in the understanding of its pathogenesis and to expand the therapeutic armamentarium.

The present study aims to evaluate the role of the combination of potassium citrate, magnesium citrate and pyridoxine to contribute to the treatment of residual microlithiasis after digital flexible ureteroscopy with Holmium laser lithotripsy. The reduction of the average size of the residual stones and their spontaneous elimination in a natural way represent the main directions of interest, but the other studied parameters are also susceptible to increased attention.

The implementation of such prophylactic measures is essential for the prevention of potential lithiasis recurrences, cost reduction and risk minimization.

The data presented may indicate a favorable direction towards a successful treatment that can be used later in the long term to combat this difficult pathology. It is worthy of consideration that the current work has achieved its stated objectives. By publishing in prestigious journals the results of the pursued directions of interest obtained during the data collection, this doctoral thesis aimed to make its contribution to the specialized literature.

7.2. Personal contributions

The descriptive analysis of the existing variables in the study revealed that there were no statistical differences between the 2 groups chosen in terms of sex, age, body mass index, location of the residual postoperative stones or the composition of the stones highlighted after crystallographic analysis.

A first conclusion was represented by the fact that already after 3 months of using the citrate combination, an improvement in the stone-free rate and the expulsion rate was

observed compared to the group that only benefited from the hygienic-dietary treatment. Also, from the point of view of reducing the size of the residual stones and increasing the urinary PH, statistically significantly favorable results were obtained for the first group.

An increase in citrate was observed in patients who managed to achieve stone-free status after 3 months more important than in the group where this was not achieved. These results were statistically significant.

Following the same line, after 6 months of treatment, favorable results were observed for the group that received the combination of citrates in terms of stone-free rate, expulsion capacity, reduction of the average size of residual stones and elevation of urinary PH.

Citrate also benefited from favorable, statistically significant results, having higher values compared to those obtained previously (at 3 months) in the group where "stone-free" status was obtained.

At 9 months of treatment, for the patients who benefited from the citrate combination, a considerably higher stone-free rate compared to the previous results was found, as well as an expulsion rate and a reduction in the mean size of the residual stones with a considerable impact compared to data obtained at 3 and 6 months, as well as an increasing urinary PH, all with statistical significance.

Next, the improvement of the citrate rate was highlighted in both groups, but with higher values for patients who no longer had residual stones, the results obtained being statistically significant.

Finally, after 12 months of treatment, a pronounced stone-free rate was found in the group in which the combination of citrates and pyridoxine was used, statistically significant, with values similar to those in the specialized literature.

The ability to expel the stones was clearly superior after 12 months to the group of patients who benefited from drug treatment along with the hygienic-dietary regimen, the results being statistically significant.

In relation to the reduction of the average size of the residual stones after 12 months, better results were obtained in the group in which citrate treatment was used than in the 2nd one, in the statistical analysis the values obtained were significant.

From the point of view of urinary PH, a statistically significant increase was found after 12 months in the group where drug treatment was used.

At 12 months of treatment, the best rates of citrate were found in both groups, respectively where the stone-free status was obtained and where this status was not acquired, but with considerably higher values for the former, the results being significant from the from a statistical point of view.

The analysis of the obtained data confirmed the usefulness of using the combination of potassium citrate, magnesium citrate and pyridoxine in the established doses to combat residual renal microlithiasis secondary to minimally invasive surgical interventions of digital flexible ureteroscopy with Holmium laser lithotripsy. The significant differences obtained give the treatment considerable advantages that can find use for the management of these cases. Also, the risk of lithiasis relapses is reduced, an aspect worth taking into account in the medium and long term.

As prospective directions, the results obtained from this work represent a good starting point, as future studies are needed to evaluate the long-term utility of this treatment in terms of improving urinary parameters and preventing stone recurrences.

Bibliography

1. Prezioso, D., et al., *Dietary treatment of urinary risk factors for renal stone formation. A review of CLU Working Group.* Archivio italiano di urologia, andrologia: organo ufficiale [di] Società italiana di ecografia urologica e nefrologica / Associazione ricerche in urologia, 2015. **87**: p. 105-120.
2. Lotan, Y., *Economics and cost of care of stone disease.* Adv Chronic Kidney Dis, 2009. **16**(1): p. 5-10.
3. Shoag, J., et al., *Risk of chronic and end stage kidney disease in patients with nephrolithiasis.* J Urol, 2014. **192**(5): p. 1440-5.
4. Pak, C.Y., *Medical management of urinary stone disease.* Nephron Clin Pract, 2004. **98**(2): p. c49-53.
5. Hamm, L.L. and K.S. Hering-Smith, *Pathophysiology of hypocitraturic nephrolithiasis.* Endocrinol Metab Clin North Am, 2002. **31**(4): p. 885-93, viii.
6. Ryall, R.L., *Urinary inhibitors of calcium oxalate crystallization and their potential role in stone formation.* World J Urol, 1997. **15**(3): p. 155-64.
7. Pak, C.Y., *Citrate and renal calculi: an update.* Miner Electrolyte Metab, 1994. **20**(6): p. 371-7.
8. Barnela, S.R., et al., *Medical management of renal stone.* Indian J Endocrinol Metab, 2012. **16**(2): p. 236-9.
9. Grasso, M. and D. Bagley, *A 7.5/8.2 F actively deflectable, flexible ureteroscope: a new device for both diagnostic and therapeutic upper urinary tract endoscopy.* Urology, 1994. **43**(4): p. 435-41.
10. Bagley, D.H., *Intrarenal access with the flexible ureteropyeloscope: effects of active and passive tip deflection.* J Endourol, 1993. **7**(3): p. 221-4.
11. Bagley, D.H., *Ureteral endoscopy with passively deflectable, irrigating flexible ureteroscopes.* Urology, 1987. **29**(2): p. 170-3.
12. Mulvaney, W.P. and C.W. Beck, *The laser beam in urology.* J Urol, 1968. **99**(1): p. 112-5.
13. Trinchieri, A., *Epidemiology of urolithiasis.* Arch Ital Urol Androl, 1996. **68**(4): p. 203-49.
14. Tiselius, H.G., *Epidemiology and medical management of stone disease.* BJU Int, 2003. **91**(8): p. 758-67.
15. Yasui, T., et al., *A replication study for three nephrolithiasis loci at 5q35.3, 7p14.3 and 13q14.1 in the Japanese population.* Journal of Human Genetics, 2013. **58**(9): p. 588-593.
16. Wimpissinger, F., et al., *The silence of the stones: asymptomatic ureteral calculi.* J Urol, 2007. **178**(4 Pt 1): p. 1341-4; discussion 1344.
17. Phillips, R., et al., *Citrate salts for preventing and treating calcium containing kidney stones in adults.* Cochrane Database Syst Rev, 2015(10): p. Cd010057.
18. Ettinger, B., et al., *Potassium-magnesium citrate is an effective prophylaxis against recurrent calcium oxalate nephrolithiasis.* J Urol, 1997. **158**(6): p. 2069-73.
19. Lojanapiwat, B., et al., *Alkaline citrate reduces stone recurrence and regrowth after shockwave lithotripsy and percutaneous nephrolithotomy.* Int Braz J Urol, 2011. **37**(5): p. 611-6.
20. Barcelo, P., et al., *Randomized double-blind study of potassium citrate in idiopathic hypocitraturic calcium nephrolithiasis.* J Urol, 1993. **150**(6): p. 1761-4.
21. Hofbauer, J., et al., *Alkali citrate prophylaxis in idiopathic recurrent calcium oxalate urolithiasis--a prospective randomized study.* Br J Urol, 1994. **73**(4): p. 362-5.
22. Iacobazzi, V. and V. Infantino, *Citrate--new functions for an old metabolite.* Biol Chem, 2014. **395**(4): p. 387-99.

23. Franklin, R.B., et al., *Evidence that Osteoblasts are Specialized Citrate-producing Cells that Provide the Citrate for Incorporation into the Structure of Bone.* Open Bone J, 2014. **6**: p. 1-7.
24. Mycielska, M.E., et al., *Extracellular Citrate in Health and Disease.* Curr Mol Med, 2015. **15**(10): p. 884-91.
25. P., D., *The Renal Handling of Citrate*, in *Urolithiasis and Related Clinical Research*, S.L.H. In: Schwille P.O., Robertson W.G., Vahlensieck W. (eds), Editor. 1985, Springer: Boston, MA.
26. Simpson, D.P., *Citrate excretion: a window on renal metabolism.* Am J Physiol, 1983. **244**(3): p. F223-34.
27. Caudarella, R. and F. Vescini, *Urinary citrate and renal stone disease: the preventive role of alkali citrate treatment.* Arch Ital Urol Androl, 2009. **81**(3): p. 182-7.
28. Granchi, D., et al., *Role of Citrate in Pathophysiology and Medical Management of Bone Diseases.* Nutrients, 2019. **11**(11): p. 2576.
29. Pak, C.Y. and M.I. Resnick, *Medical therapy and new approaches to management of urolithiasis.* Urol Clin North Am, 2000. **27**(2): p. 243-53.
30. Vallés, P.G. and D. Batlle, *Hypokalemic Distal Renal Tubular Acidosis.* Adv Chronic Kidney Dis, 2018. **25**(4): p. 303-320.
31. Goraya, N., et al., *Urine citrate excretion as a marker of acid retention in patients with chronic kidney disease without overt metabolic acidosis.* Kidney Int, 2019. **95**(5): p. 1190-1196.
32. Chow, K., et al., *Citrate inhibits growth of residual fragments in an in vitro model of calcium oxalate renal stones.* Kidney Int, 2004. **65**(5): p. 1724-30.
33. Astroza, G.M., et al., *Treatment Response in Patients with Stones, and Low Urinary pH and Hypocitraturia Stratified by Body Mass Index.* J Urol, 2016. **195**(3): p. 653-7.
34. Skolarikos, A., G. Alivizatos, and J. de la Rosette, *Extracorporeal shock wave lithotripsy 25 years later: complications and their prevention.* Eur Urol, 2006. **50**(5): p. 981-90; discussion 990.
35. Geavlete, P.A., et al., *Chapter 6 - Retrograde Ureteroscopy in the Treatment of Upper Urinary Tract Lithiasis*, in *Retrograde Ureteroscopy*, P.A. Geavlete, Editor. 2016, Academic Press: San Diego. p. 105-216.
36. Ferraro, P.M., et al., *Risk of Kidney Stones: Influence of Dietary Factors, Dietary Patterns, and Vegetarian-Vegan Diets.* Nutrients, 2020. **12**(3).
37. Boothby, W.M. and M. Adams, *THE OCCURRENCE OF CITRIC ACID IN URINE AND BODY FLUIDS.* American Journal of Physiology-Legacy Content, 1934. **107**(2): p. 471-479.
38. Kissin, B. and M.O. Locks, *Urinary Citrates in Calcium Urolithiasis.* Proceedings of the Society for Experimental Biology and Medicine, 1941. **46**(2): p. 216-218.
39. Huang, Z.J., et al., *[Study on nano- and microcrystallites in the urines of calcium oxalate stone formers].* Guang Pu Xue Yu Guang Pu Fen Xi, 2010. **30**(7): p. 1913-7.
40. Duan, C.Y., et al., *Changes in urinary nanocrystallites in calcium oxalate stone formers before and after potassium citrate intake.* Int J Nanomedicine, 2013. **8**: p. 909-18.
41. Prezioso, D., et al., *Dietary treatment of urinary risk factors for renal stone formation. A review of CLU Working Group.* Arch Ital Urol Androl, 2015. **87**(2): p. 105-20.
42. Koenig, K., et al., *Bioavailability of potassium and magnesium, and citraturic response from potassium-magnesium citrate.* J Urol, 1991. **145**(2): p. 330-4.
43. Rodgers, A., S. Allie-Hamdulay, and G. Jackson, *Therapeutic action of citrate in urolithiasis explained by chemical speciation: increase in pH is the determinant factor.* NEPHROLOGY DIALYSIS TRANSPLANTATION, 2006. **21**(2): p. 361-369.
44. Conte Visús, A., et al., *[Biochemical effects of potassium citrate in the treatment of calcium oxalate lithiasis].* Arch Esp Urol, 1994. **47**(2): p. 141-50.
45. Coll, D.M., M.J. Varanelli, and R.C. Smith, *Relationship of Spontaneous Passage of Ureteral Calculi to Stone Size and Location as Revealed by Unenhanced Helical CT.* American Journal of Roentgenology, 2002. **178**(1): p. 101-103.

46. Segura, J.W., et al., *Ureteral Stones Clinical Guidelines Panel summary report on the management of ureteral calculi. The American Urological Association.* J Urol, 1997. **158**(5): p. 1915-21.
47. Pak, C.Y., et al., *Long-term treatment of calcium nephrolithiasis with potassium citrate.* J Urol, 1985. **134**(1): p. 11-9.
48. Spivacow, F.R., et al., *Long-term treatment of renal lithiasis with potassium citrate.* Urology, 2010. **76**(6): p. 1346-9.
49. Elbaset, M.A., et al., *Optimal non-invasive treatment of 1-2.5 cm radiolucent renal stones: oral dissolution therapy, shock wave lithotripsy or combined treatment-a randomized controlled trial.* World J Urol, 2020. **38**(1): p. 207-212.
50. Rosa, M., et al., *Recent finding and new technologies in nephrolithiasis: a review of the recent literature.* BMC Urology, 2013. **13**(1): p. 10.