



## Assessment of constituents of the renal calculi in affected patients

Saiqa Rasool Memon<sup>1</sup>, Gul Afshan Soomro<sup>1</sup>, Ghulam Abbas Shar<sup>\*1</sup>,  
Muhammad Bachal Korai<sup>1</sup>, Nisar Abbas Shar<sup>2</sup>

<sup>1</sup>*Institute of Chemistry, Shah Abdul Latif University, Khairpur, Sindh, Pakistan*

<sup>2</sup>*Institute of Microbiology, Shah Abdul Latif University, Khairpur, Sindh, Pakistan*

Article published on July 30, 2020

**Key words:** Renal calculi, Calculi composition, Cystine calculi, FT-IR

### Abstract

Renal calculi illness is also referred to as urolithiasis or nephrolithiasis. Urolithiasis has become the third most frequent medical disorder globally. Pakistan has probably the most significant incidence of kidney stones. The detection of stone components is most important, since it gives the data which may be helpful to health professionals in finding the main cause of renal calculi and deciding whether they should be treated in a therapeutic or surgical manner. The purpose of present work was to assess the constituents of renal calculi removed via laparoscopic surgery from the affected persons in the Peoples University of Medical & Health Sciences for Women, Shaheed Benazir Abad, Nawab Shah, Sindh, Pakistan. The renal stones were assessed by FT-IR for their constitutional study. Out of 60 investigated specimen of renal calculi, 32/60 (53.3%) were calcium oxalate, 12/60 (20%) were calcium phosphate, 09/60 (15%) were uric acid calculi, 04/60 (6.7%) were struvite and cysteine calculi were 03/60 (5%). The current study showed that the calcium oxalate calculi were the most prevalent (53.3%) while the cysteine calculi were the lowermost prevalent (5%) among the all collected and examined calculi.

**\*Corresponding Author:** Ghulam Abbas Shar ✉ [gabbas.shar@salu.edu.pk](mailto:gabbas.shar@salu.edu.pk)

## Introduction

Renal calculi illness is also referred to as urolithiasis or nephrolithiasis (Gottlieb *et al.*, 2018; Vasudevan *et al.*, 2017). Urolithiasis has become the third most frequent medical disorder globally (Farooq *et al.*, 2018) affecting up to fifteen percent of the Western countries' population (Banov and Ceban, 2017). Pakistan has probably the most significant incidence of kidney stones (Samad *et al.*, 2017). Renal calculi often consist of chemicals like magnesium, ammonium, calcium, uric acid and some other salts dissolved in the urine at normal levels. If these salts increase in concentration, they become crystals and supersaturated and rise to develop renal calculi (Alelign and Petros, 2018). The chemical components that are most common in urolithiasis involve calcium (75% to 80%) followed by calcium phosphate and calcium oxalate. 10% to 15% of the components contain phosphate, ammonium, struvite and magnesium. The 6% is comprised of uric acid and merely 1-2% contains cystine (Shokouhi *et al.*, 2008). Renal calculi are typically formed in kidney and exit from the body via the urinary tract. Little calculi can pass easily causing no symptoms. When a stone expands to over 5 mm (0.2 inch), it may cause the ureter to block, leading to intense lower back or abdominal pain (Ansari *et al.*, 2017).

Renal calculi disease is a recurring disorder (Zisman, 2017). People who suffer from kidney stones that had one stone will develop to another (Riordan *et al.*, 2009). Stones produce twice in men and once in women. Once a kidney stone formed, the chance of second stone formation is approximately 50% within five to seven years (Parmar, 2004). This disorder has a multifactorial origin and is affected by the physico-chemical status of the urinary tract (Daudon *et al.*, 1993). Gender, diet, heredity, age and weather are severe aspects of risk (Wróbel & Kuder, 2019). Renal calculi is in fact a disorder of every age (Shabsoug *et al.*, 2016). Different tests may indicate the presence of kidney stones. Physical tests may reveal cramping pains in the groin and the lower portion near the kidneys. It is often a warning of disorder. A urinalysis will indicate whether or not there is blood in the urine

and if there is a subsequent infection (Simerville *et al.*, 2005). A method to check the kidney stones is CT scans of the abdominal cavity. Computed tomography would assess the ureter, bladder and kidney condition, the existence or lack of a stone, the precise sizes and locations of the renal stone, the existence or lack of an obstruction and the status of the organs in the region like the aorta, appendix and pancreas (Brisbane *et al.*, 2016). The ultrasound also showed good results of screening and can identify various complications associated with kidney stones (Ganesan *et al.*, 2017). Once a stone is detected in a person, X-rays are simply used to monitor the growth of the stone through a urinary system.

The detection of stone components is most important, since it gives the data which may be helpful to health professionals in finding the main cause of renal calculi and deciding whether they should be treated in a therapeutic or surgical manner (Channa *et al.*, 2007). The treatment of urinary stone can be painful, stone removal often requires surgery, and renal failure occurs in about 3% of patients. Medicinal prevention is broadly acknowledged and advised for recurrent nephrolithiasis patients (Qaseem *et al.*, 2014). For those with stones, prevention consists of consuming liquids in order to produce over 2 liters urine in a day (Heilberg and Schor, 2006). In case it is not sufficiently useful, one can take thiazide diuretic, citrate or allopurinol (Fink *et al.*, 2013). Patients with kidney stones should be treated either surgically or medically (Shafi *et al.*, 2016). The purpose of treating them is the removal of the stone, the elimination of infection, the protection of renal function and the stopping of reoccurrence (Xu *et al.*, 2013).

Numerous analytical methods are available for stone analysis, which include X-ray powder diffraction (Saçlı *et al.*, 2019), dry and wet spots tests (Khan *et al.*, 2018), Raman spectroscopy (Cui *et al.*, 2018) and Fourier Transform Infrared spectroscopy (FT-IR) (D'Alessandro *et al.*, 2017). The FT-IR spectrometry was introduced for the first time in 1955. Due to its uniqueness and rapidity, it quickly gained the most prominent reference technique for analyzing stone.

In terms of results and accuracy, FTIR has an advantage compared to conventional methods (Dao NQ, Daudon M. 1997; Khaskheli *et al.*, 2012). The most important benefit of FT-IR in analyzing kidney stones is the rapidity to identify different stones. Hence in our current research study we also employed the FT-IR technique for the constitutional assessment of the renal calculi in affected persons.

**Materials and methods**

*Frequency of subject*

Sixty (60) renal calculi specimens were collected from Peoples University of Medical and Health Sciences for Women, Shaheed Benazir Abad, Nawab Shah, Sindh, Pakistan. Out of these sixty specimens, forty five (45) were specimens of males and fifteen (15) were of females. Their age ranges from 20 to 80 years as presented in Table 3 and Fig. 4-5. And the ratio of males to females was 3:1. All the affected persons were from the same district Shaheed Benazir Abad, Nawab Shah, Sindh, Pakistan and had lower socioeconomic background. They consumed locally and easily available food like different types of vegetables, pulses etc. and meat in least quantity.

*Examination of calculi specimens*

This study was performed on renal calculi that were eliminated with the surgery of 60 people affected in the years 2018 to 2019. Whole the 60 collected renal calculi were placed on sterilized gauze to dry in the air and then carefully cleaned using double-distilled H<sub>2</sub>O (in order to remove the organic materials) and dried on 37°C in an oven appropriately, after which the samples' weight was calculated. After studying the morphological features like shapes, colours and sizes,

each patient's specimens of renal calculus were carefully divided into four equal parts and one part from them was crushed using a pestle and mortar. Afterwards, the thin, uniform powders of the calculi specimens were kept in a test-tube, preserved in a dark place until they were analyzed. At the end, the composition of the powdered stones was assessed with the FT-IR spectrometer.

*FT-IR spectroscopy*

The FT-IR technique is a highly favoured and broadly employed technique for the investigation of urolithiasis. It is a suitable analytical technique for the assessment of composition of renal calculi. The technique is speedy and reliable and provides both semi-quantitative and qualitative analysis. In addition, measurement of relevant quantities of each component available is possible with no use of solvents. The advantages of FT-IR as compared to dispersive procedures are its mechanical ease, rapidity, internal calibration, precision and quality control operations.

**Results and discussion**

The morphological examination of specimens depended on the observation of the physical features of the stones, such as shape, weight, texture and colour. Every specimen was examined with great care and distinguished by its physical appearance. It was observed that the samples of calculi were different in shape like round, oval, triangle etc. as shown in Table 1 and Fig. 1, also were in different colours like white, black, brown, yellow etc. as shown in Table 1 and Fig. 2 and were of different weights ranges from 0-1g to 4-5g as shown in Table 1 and Fig. 3.

**Table 1.** Morphological characteristics shapes, colours and weights of the renal calculi samples.

Shapes of the renal calculi samples						
Types of renal calculi	Shapes					
	Round	Irregular	Oval	Staghorn	Cubical	Triangular
Calcium Oxalate	09	01	15	03	02	02
Calcium Phosphate	03	00	06	02	00	01
Uric Acid	04	02	03	00	00	00
Struvite	00	01	01	02	00	00
Cystine	01	01	01	00	00	00
Colours of the renal calculi samples						
Types of renal calculi	Colours					
	White	Black	Yellow	Brown	Off white	
Calcium Oxalate	09	07	03	08	05	

Shapes of the renal calculi samples					
Calcium Phosphate	04	00	00	02	06
Uric Acid	00	03	05	00	01
Struvite	02	00	00	01	01
Cystine	00	00	01	02	00
Weights of the renal calculi samples					
Types of renal calculi	Weights				
	0-1g	1-2g	2-3g	3-4g	4-5g
Calcium Oxalate	03	09	15	04	01
Calcium Phosphate	00	00	02	06	04
Uric Acid	03	01	04	01	00
Struvite	00	01	01	00	02
Cystine	00	02	00	01	00

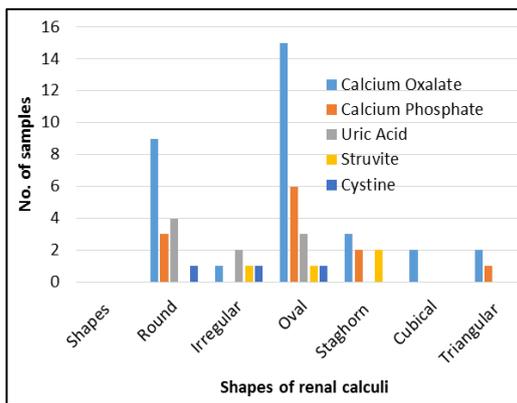


Fig. 1. Shapes of the renal calculi samples.

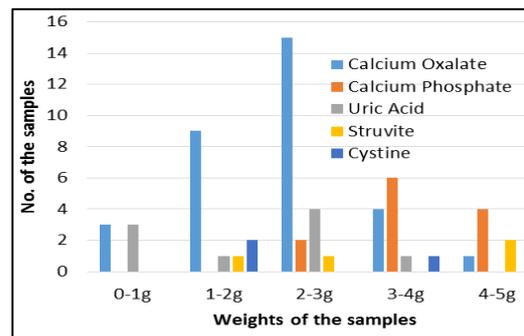


Fig. 3. Weights of the renal calculi samples.

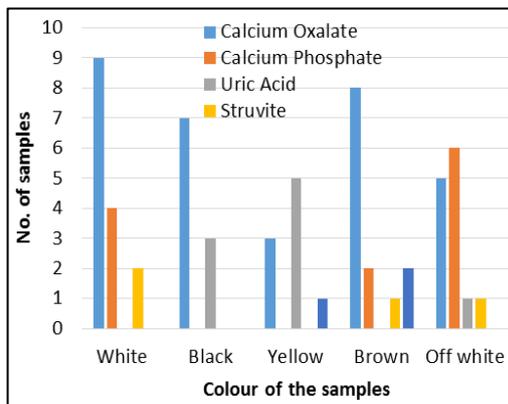


Fig. 2. Colours of the renal calculi samples.

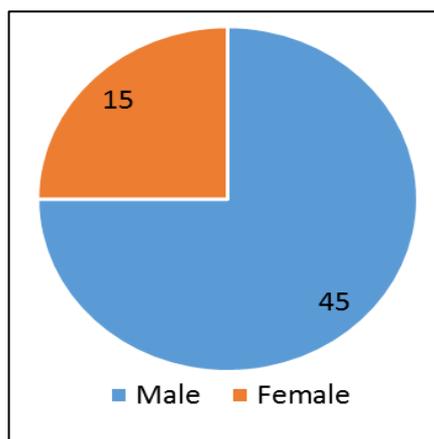
Among 60 samples taken from people suffering from urolithiasis, 15 (25%) were females and 45 (75%) males and the ratio of males to females was 3:1 as shown in Table 3 and Fig. 4 ages range 20 to 70, with predominance of the ratio of males over females, and the majority of kidney stones cases were seen from 30 to 48 years of age as shown in Table 3 and Fig. 5. The disease was observed to be more common in the 31-48 age group 30/60 (50%) followed by 20-30 years 16/60 (26.7%), 49-60 years 11 (18.3%) and 61-70 years 3/60 (5%). And the disease was observed to be least in the 61-70 age group 3/60 (5%) as shown in Table 3 and Fig. 5.

Table 2. Prevalence of FTIR bands of renal calculi samples.

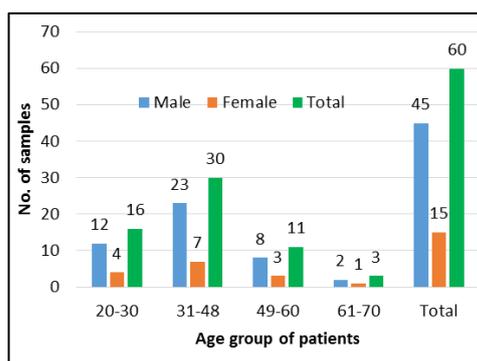
Type of renal calculi	No & percentage of samples	Observed FT-IR bands (in cm <sup>-1</sup> )	In literature (cm <sup>-1</sup> )
Calcium oxalate	32 (53.3%)	3049, 3058, 1619, 1321, 776, 665, 517	3060, 1618, 1312, 782, 667, 514 [Sekkoum <i>et al.</i> , 2016]
Calcium Phosphate	12 (20%)	3465, 1459, 1026, 505-781	3429, 2800, 1469, 1419, 1316, 1038, 872, 781, 606 [Wilson <i>et al.</i> , 2010]
Uric acid	09 (15%)	3012, 2810, 2669, 2597, 1670, 1593, 1399, 1111	3142, 3026, 2857, 1353, 1126, 1031, 788 [Sofia <i>et al.</i> , 2010]
Struvite	04 (6.7%)	2864, 1583, 1424, 1041, 869	2362.67, 1459.29 [Channa <i>et al.</i> , 2007]
Cystine	03 (5%)	3035, 1599, 1473, 835, 541	3026, 1618.28, 1485, 846.75 [Marickar <i>et al.</i> , 2009]

**Table 3.** Incidence and classification of renal calculi age and sex-wise of the affectless.

Calculi based on the age groups					
Age in groups	20-30	31-48	49-60	61-70	Total
Male	12	23	08	02	45
Female	04	07	03	01	15
Total	16	30	11	03	60
%	26.7%	50%	18.3%	5%	100%
Sex-wise classification					
Gender	Male	Female	Total		
Number of patients	45	15	60		
%	75%	25%	100%		
Male: Female Ratio	3:1				



**Fig. 4.** Classification of renal calculi based on the sex-wise.



**Fig. 5.** Age-wise classification of renal calculi.

The current study showed that the calcium oxalate calculi were the most prevalent among the analyzed stones. Out of 60 investigated specimen of renal calculi, 32/60 (53.3%) were calcium oxalate, 12/60 (20%) were calcium phosphate, 09/60 (15%) were uric acid calculi, 04/60 (6.7%) were struvite and cystine calculi were 03/60 (5%) as shown in Table 2.

The prevalence of cystine calculi 03/60 (5%) was the lowest among the all collected and examined calculi. FT-IR spectroscopy, due to its uniqueness and rapidity, quickly gained the most prominent reference technique for analyzing stone. In terms of results and accuracy, FTIR has an advantage compared to conventional methods (Dao NQ, Daudon M. 1997; Khaskheli *et al.*, 2012). The most important benefit of FT-IR in analyzing kidney stones is the rapidity to identify different stones. Hence in our current research study we also employed the FT-IR technique for the constitutional assessment of the renal calculi in affected persons admitted in the Peoples University of Medical & Health Sciences for Women, Shaheed Benazir Abad, Nawab Shah, Sindh, Pakistan.

The specimens were examined and assessed according to their FTIR spectrum in relation to the standards. The calcium oxalate is the major constituent of all renal calculi. The pure calcium oxalate has been characterized with 5 bands from 3049cm<sup>-1</sup> to 3058cm<sup>-1</sup>, because of the symmetrical and asymmetrical elongation of O-H, absorption higher at 1619cm<sup>-1</sup> and 1321cm<sup>-1</sup> corresponding to C=O and C-O respectively. There are bands at 776cm<sup>-1</sup> and 665cm<sup>-1</sup> that correspond to the mode of bending of C-H and outside the plane of bending of O-H.

In the bending plane O-C=O is formed at 517cm<sup>-1</sup> as shown in Table 2. Stretching frequencies of 2864, 1583, 1424, 869 and 1041cm<sup>-1</sup> revealed corresponding with struvite calculi. Bands for cystine calculi was observed at 3035, 1599, 1473, 835 and 54cm<sup>-1</sup> as shown in Table 2. Bands for Calcium phosphate were observed at 3465 cm<sup>-1</sup> that is because of symmetrical and asymmetrical stretching of O-H. The stretching vibration of phosphine group P=O were observed at 1459cm<sup>-1</sup>, higher peak of 1026cm<sup>-1</sup> assigned to O-P of the PO<sub>4</sub><sup>3-</sup> groups, plus other outside the plane deformations (O-P-O and O=P-O) of PO<sub>4</sub><sup>3-</sup> groups appeared at 505-781cm<sup>-1</sup> as shown in Table 2.

The uric acid calculi can easily be known via the occurrence of various characteristic stretching bands of N-H ranges 2590-3600cm<sup>-1</sup>.

Two bands were observed at  $1593\text{cm}^{-1}$  and  $1670\text{cm}^{-1}$  corresponding to carbonyl of conjugated amide and carbonyl (C=O) of urea groups respectively. The four bands were observed at  $2597$ ,  $2669$ ,  $2810$  and  $3012\text{cm}^{-1}$  conforming the stretching of N-H, and other bands attributed to hydrogen bonding that appear in the range  $3100\text{-}3500\text{cm}^{-1}$ . The characteristic stretching band appeared at  $1399\text{cm}^{-1}$  due to carbonyl and amide group hypsochromic effect. The vibrational bands of =C-N and O=C-N were found to be appeared at  $1139\text{cm}^{-1}$  and  $1111\text{cm}^{-1}$  respectively as shown in Table 2. Bands for components of renal calculi samples were in conformity with the literature reported as shown in Table 2.

### Conclusion

The current study concluded that the calcium oxalate calculi were the most prevalent (53.3%) among the all the analyzed renal calculi removed via laparoscopic surgery from the affected persons in the Peoples University of Medical & Health Sciences for Women, Shaheed Benazir Abad, Nawab Shah, Sindh, Pakistan. The renal calculi disease was observed to be more common in the 30-48 age group (50%). The ratio of males to females was found as 3:1.

### References

**Alelign T, Petros B.** 2018. Kidney stone disease: an update on current concepts. *Advances in urology* **2018**, 1-12.

**Ansari A, Singh SP, Khinchi MP, Shama P, Mahaver M.** 2017. A brief review on: Kidney stone. *Asian Journal of Pharmaceutical Research and Development* **5**, 1-9.

**Banov P, Ceban E.** 2017. The efficacy of metaphylaxis in treatment of recurrent urolithiasis. *Journal of medicine and life* **10**, 188-193.

**Brisbane W, Bailey MR, Sorensen MD.** 2016. An overview of kidney stone imaging techniques. *Nature reviews urology* **13**, 654-662.

**Channa NA, Ghangro AB, Soomro AM, Noorani L.** 2007. Analysis of kidney stones by FTIR Spectroscopy. *Jlumhs* **2**, 66-73.

**Cui X, Zhao Z, Zhang G, Chen S, Zhao Y, Lu J.** 2018. Analysis and classification of kidney stones based on Raman spectroscopy. *Biomedical Optics Express* **9**, 4175-4183.

**D'Alessandro MM, Gennaro G, Tralongo P, Maringhini S.** 2017. Fourier Transform Infrared Analysis of Urinary Calculi and Metabolic Studies in a Group of Sicilian Children. *Iranian Journal of Kidney Diseases* **11**, 209-216.

**Dao NQ, Daudon M.** 1997. Infrared and Raman spectra of calculi. Elsevier.

**Daudon M, Bader CA, Jungers P.** 1993. Urinary calculi: review of classification methods and correlations with etiology. *Scanning Microscopy* **7**, 1081-1106.

**Farooq MU, Mustafa SH, Shah MT, Khan MJ, Iftikhar O.** 2018. Dietary and fluid intake habits in nephrolithiasis patients presented to Ayub Teaching Hospital, Abbottabad. *International Journal* **4**, 274.

**Fink HA, Wilt TJ, Eidman KE, Garimella PS, MacDonald R, Rutks IR, Brasure M, Kane RL, Ouellette J, Monga M.** 2013. Medical management to prevent recurrent nephrolithiasis in adults: a systematic review for an American College of Physicians Clinical Guideline. *Annals of Internal Medicine* **158**, 535-543.

**Ganesan V, De S, Greene D, Torricelli FCM, Monga M.** 2017. Accuracy of ultrasonography for renal stone detection and size determination: is it good enough for management decisions. *Bju International* **119**, 464-469.

**Gottlieb M, Long B, Koyfman A.** 2018. The evaluation and management of urolithiasis in the ED: A review of the literature. *The American Journal of Emergency Medicine* **36**, 699-706.

**Heilberg IP, Schor N.** 2006. Renal stone disease: causes, evaluation and medical treatment. *Arquivos Brasileiros de Endocrinologia & Metabologia* **50**, 823-831.

- Khan AH, Imran S, Talati J, Jafri L.** 2018. Fourier transform infrared spectroscopy for analysis of kidney stones. *Investigative and clinical urology* **59**, 32-37.
- Khaskheli MH, Sherazi STH, Ujan HM, Mahesar SA.** 2012. Transmission FT-IR spectroscopic analysis of human kidney stones in the Hyderabad region of Pakistan. *Turkish Journal of Chemistry* **36**, 477-483.
- Marickar YF, Lekshmi PR, Varma L, Koshy P.** 2009. Problem in analyzing cystine stones using FTIR spectroscopy. *Urological research* **37**, 263-269.
- Qaseem A, Dallas P, Forcica MA, Starkey M, Denberg TD.** 2014. Dietary and pharmacologic management to prevent recurrent nephrolithiasis in adults: a clinical practice guideline from the American College of Physicians. *Annals of Internal Medicine* **161**, 659-667.
- Riordan HD, Casciari JJ, Gonzalez MJ, Riordan NH, Miranda-Massari JR, Taylor P, Jackson JA.** 2009. A pilot clinical study of continuous intravenous ascorbate in terminal cancer patients. *Puerto Rico health Sciences Journal* **24**, 267-276.
- Saçlı B, Aydınalp C, Cansız G, Joof S, Yılmaz T, Çayören M, Önal B, Akduman I.** 2019. Microwave dielectric property based classification of renal calculi: Application of a kNN algorithm. *Computers in Biology and Medicine* **112**, 103366-103373.
- Samad N, Liaqat S, Anwar M, Tehreem K, Sadiq HM.** 2017. Chemical nature of various types of renal stones in the population of district Multan Pakistan. *Pakistan Journal of Pathology* **28**, 56-60.
- Sekkoum K, Cheriti A, Taleb S, Belboukhari N.** 2016. FTIR spectroscopic study of human urinary stones from El Bayadh district (Algeria). *Arabian Journal of Chemistry* **9**, 330-334.
- Shabsoug BM, AL-Quraishi AY, AL-Mgassgas HM.** 2016. Chemical Analysis of Kidney Stones in Northern Jordan. *Journal of Applicable Chemistry* **5**, 719-726.
- Shafi H, Moazzami B, Pourghasem M, Kasaecian A.** 2016. An overview of treatment options for urinary stones. *Caspian journal of internal medicine* **7**, 1-6.
- Shokouhi B, Gasemi K, Norizadeh E.** 2008. Chemical composition and epidemiological risk factors of urolithiasis in Ardabil Iran. *Research Journal of Biological Sciences* **3**, 620-626.
- Simerville JA, Maxted WC, Pahira JJ.** 2005. Urinalysis: a comprehensive review. *American family physician* **71**, 1153-1162.
- Sofia PG, Ionescu I, Rodica G, Anisoara P.** 2010. The use of infrared spectroscopy in the investigation of urolithiasis. *Revista română de medicină de laborator* **18**, 67-77.
- Vasudevan V, Samson P, Smith AD, Okeke Z.** 2017. The genetic framework for development of nephrolithiasis. *Asian journal of urology* **4**, 18-26.
- Wilson EV, Bushiri MJ, Vaidyan VK.** 2010. Characterization and FTIR spectral studies of human urinary stones from Southern India. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* **77**, 442-445.
- Wróbel G, Kuder T.** 2019. The role of selected environmental factors and the type of work performed on the development of urolithiasis—a review paper. *International Journal of Occupational Medicine and Environmental Health* **32**, 761-775.
- Xu H, Zisman AL, Coe FL, Worcester EM.** 2013. Kidney stones: an update on current pharmacological management and future directions. *Expert Opinion on Pharmacotherapy* **14**, 435-447.
- Zisman AL.** 2017. Effectiveness of treatment modalities on kidney stone recurrence. *Clinical Journal of the American Society of Nephrology* **12**, 1699-1708.