



Evaluation of the components of the kidney stone samples

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Abstract

Kidneys are a couple of bean-like organs that are present in vertebrates. When blood is passed through kidney, it filters wastes, unnecessary water and chemicals out of the blood. Kidneys extract the by-products of the blood and generate urine. Kidneys control the level of fluids, salts, minerals, and other materials in the organisms. The kidneys can form kidney stones as the balance of such substances alters. Pakistan has probably the most significant incidence of kidney stones. The main aim of current research was to evaluate the components of kidney stones. The FTIR technique was employed for the evaluation of the components of kidney stones. Fifty five (55) kidney calculi samples were collected and examined. From these 55 examined kidney stones samples, 31(56.4%) were calcium oxalate, 11(20%) calcium phosphate, 08(14.5%) uric acid, 03(5.5%) struvite and 02(3.6%) cysteine stones. The kidney stone disease was most frequently observed 31(56.4%) in the age group 26-45 years. The male to female ratio was found as 4:1 (M:F) ratio.

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Introduction

Kidneys are a couple of bean-like organs that are present in vertebrates. These are situated at the left and right side of the retroperitoneal space and these are about 12 centimeters long in adults (Lote, 2016; Mescher, 2016). Every kidney is connected to the ureter, the tube which transports the excreted urine into the bladder. Kidneys extract the by-products of the blood and generate urine. When blood is passed through kidneys, it filters wastes, unnecessary water and chemicals out of the blood (Tyagi, 2019). Hormones are also produced by the kidneys to assist in controlling blood pressure and stimulate bone marrow to form erythrocytes. The nephrology is a clinical specialty that deals with disorders of renal function such as acute renal lesions, nephrotic and nephritic syndromes, pyelonephritis and chronic kidney disorders. The urology deals with disorders of the anatomy of the kidneys (and urinary tract) which include tumours, kidney cysts, ureteral and renal calculi and obstruction of the urinary tract (Hoda and Hoda, 2005). Kidneys control the level of fluids, salts, minerals, and other materials in the organism. The kidneys can form kidney stones as the balance of such substances alters.

Kidney calculi mostly consist of chemicals like magnesium, calcium, ammonium, uric acid and other salts dissolved within the urine at their normal concentration. Such salts transform themselves in crystals when their concentration increases and are then saturated and develop into kidney calculi (Alelign and Petros, 2018; Shabsoug *et al.*, 2016). The seventy-five percent of renal calculi that go to the kidney stone analysis laboratory contains calcium oxalate as a dominant constituent (Musa and Idris, 2017). The urolithiasis has been a worldwide problem that has affected people for several decades (Subhan *et al.*, 2014). In different parts of the globe, the total number of stones creating opportunities for kidney stones varies and recurrence rate of kidney calculi is estimated to be 75% in 20 years, 1-5% in Asia, 5-9% in Europe and 13% in North America. The number of kidney stones in the USA has increased over the last 30 years (Samad *et al.*, 2017).

The most common chemical components of kidney stones are calcium (75% to 80%), followed by calcium phosphate and calcium oxalate. Between 10%-15% of the ingredients comprise struvite, ammonium, phosphate and magnesium. The 6% consists of uric acid and just 1%-2% consists of cysteine (Altaf *et al.*, 2013; Shokouhi *et al.*, 2008). The main purpose of the current research work is to evaluate the chemical composition and detect the prevalence of different types of kidney stones. Evaluation of the constituents of kidney stones is essential as it provides information to physicians whether patients should be treated surgically or therapeutically (Channa *et al.*, 2007; Shafi *et al.*, 2016) for the removal of the stones, the elimination of infection, the protection of renal function and the stopping of reoccurrence (Xu *et al.*, 2013). In Pakistan, the kidney stone disease is one of the most prevalent. Because of the rising number of stone sufferers and the complications of this problem, it is necessary to study this major disease.

In many cases, renal stones are not painful until after they pass through the kidneys, along the ureter, and to the bladder. According to the stone's size, its passage along the urinary tract may produce intense pain with immediate onset of the stone (Ansari *et al.*, 2017). Some of the signs and symptoms of renal calculi are bleeding in the urine and abdominal pain, flank, or groin. People who have had one stone may progress to another (Riordan *et al.*, 2009). Urolithiasis is among the most painful illnesses that life can bring about. Recent investigations worldwide have shown that the incidence of the urolithiasis has been rising for approximately half of a century (Sekkoum *et al.*, 2016). It is a multi-factorial disease that is affected by both the chemical and physical conditions of the urinary systems (Singh and Rai, 2014). The known factors that raise the risk of developing stones in the kidney involve nutrition, dehydration, heredity, climate and the existence of certain health disorders (Wróbel & Kuder, 2019). Renal calculi is in fact a disorder at any age (Shabsoug *et al.*, 2016). Identifying the constituents of kidney stones is essential, because it gives information which can be helpful to physicians in finding out what the

primary cause of kidney stones is and in deciding whether kidney stone patients should be treated surgically or therapeutically (Channa *et al.*, 2007; 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). There are many different methods of analysis for analyzing stone, including Energy dispersive X-ray fluorescence technique (Ekinci and Şahin, 2002), dry and wet spots tests (Khan *et al.*, 2018), X-ray powder diffraction (Saçlı *et al.*, 2019), Raman spectroscopy (Cui *et al.*, 2018), laser-induced breakdown spectroscopy (Singh *et al.*, 2009) and Fourier Transform Infrared spectroscopy (FT-IR) (D'Alessandro *et al.*, 2017). The FT-IR spectrometry quickly gained the most prominent reference technique for analyzing stone. In terms of performance and accuracy, FTIR spectrometry has advantages over conventional methods (Dao and Daudon, 1997; Khaskheli *et al.*, 2012).

The best technique for the kidney stones analysis is sensitive, selective, precise, accurate, quick and reasonably en-expensive. The most significant and beneficial technique than the others is FT-IR for kidney stone analysis because it is the fast, least quantity of samples needed and fraction of a minute required for identification of different types of the constituents simultaneously from the single sample of kidney stones. Therefore, in this study we used the FT-IR technique also for the evaluation of constituents of kidney stones.

Materials and methods

Frequency of subject

Fifty five (55) kidney calculi samples were collected. Out of these fifty five, forty four (44) were specimens of males and eleven (11) were of females. Their age ranges from 15 to 72 years. And the ratio of males to females was 4:1. All the affected persons were with same lower socioeconomic background.

Examination of calculi specimens

This study was performed on renal calculi that were eliminated with the surgery of 55 affected people.

Whole the 55 collected renal calculi were placed on sterilized gauze to dry in the air and then carefully cleaned using double-distilled H₂O and dried on 37°C in an oven appropriately. 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.

Results and discussion

In our current study, the samples were tested and evaluated in accordance with their FTIR spectrum with respect to the standards. Pure calcium oxalate was characterized by main bands from 3050cm⁻¹ to 3055cm⁻¹, because of O-H symmetrical and asymmetrical elongation, higher absorption at 1623cm⁻¹ and 1319cm⁻¹ which corresponds to C=O and C-O respectively. Bands at 781cm⁻¹ and 671cm⁻¹ appeared which corresponded to the bending mode of C-H and O-H outside the bending plane. O-C=O band formed at 515cm⁻¹ in its bending plane as shown in Table 1. The 2858, 1581, 1456, 871 and 1052cm⁻¹ stretching frequencies revealed corresponding with struvite stones. The uric acid stones can easily be recognized by the appearance of different characteristics bands of stretching N-H ranges 2590-3600cm⁻¹. The 1587cm⁻¹ and 1673 cm⁻¹ two bands were observed and corresponded to carbonyl of conjugated amide and urea groups respectively. The bands at 2599, 2673, 2860 and 3028cm⁻¹ were observed conforming N-H stretching, and other at 3100-3500cm⁻¹ attributed to hydrogen bonding. The stretching band at 1357cm⁻¹ due to hypsochromic effect of carbonyl and amide groups. The 1112cm⁻¹ and 1051cm⁻¹ vibrational bands were appeared for =C-N and O=C-N respectively as shown in Table 1. The 3029, 1619, 1479, 843 and 538cm⁻¹ bands were observed for cystine stones. The bands at 3431 cm⁻¹ were observed for Calcium phosphate which were due to O-H symmetrical and asymmetrical stretching.

The 1467 cm⁻¹ band was observed for phosphine group P=O stretching vibration, the 1034 cm⁻¹ higher peak was assigned to O-P of the PO₄⁻³ group and others 503-781 cm⁻¹ appeared for outer the plane deformations (O-P-O and O=P-O) of PO₄⁻³ group as shown in Table 1. The bands for components of kidney stone sample were according to the literature reported as shown in Table 1. Current research has shown that calcium oxalate was the most common among the stones analyzed. Of the 55 kidney stone samples examined, 31(56.4%) were calcium oxalate, 11(20%) calcium phosphate, 08(14.5%) uric acid, 03(5.5%) struvite and 02 (3.6%) cystine stones as shown in Table 1.

The cysteine stones were least prevalent 02 (3.6%) amongst the whole studied stones. Of the 55 samples collected from patients with urolithiasis, 44(80%) were males and 11 (20%) females as shown in Fig. 1. and 1:4 ratio was found for the females to males ratio as shown in Table 2 and their age varied between 15-72 years, with men predominating over women, and most cases of kidney stones were observed at 26-45 years, as shown in Table 2. The disease was most frequently observed in the age group 26-45 years 31 (56.4%), then 15-25 years 13(23.6%), 46-55 years 08 (14.5%), and 56-72 years 03 (5.5%). The lowest number of patients was in the age group 56-72 years 03(5.5%), which is given in Table 2.

Table 1. Occurrence and FT-IR bands observed in kidney stone samples.

No. & % of samples	Type of renal calculi	Literature reported (cm ⁻¹)	FT-IR bands observed in present study (cm ⁻¹)
31 (56.4%)	Calcium oxalate	3060, 1618, 1312, 782, 667, 514 [Sekkoum <i>et al.</i> , 2016]	3050, 3055, 1623, 1319, 781 671, 515
11 (20%)	Calcium Phosphate	872, 781, 606 [Wilson <i>et al.</i> , 2010]	3431, 1467, 1034, 503-781
08 (14.5%)	Uric acid	3142, 3026, 2857, 1353, 1126, 1031, 788 [Sofia <i>et al.</i> , 2010]	3028, 2860, 2673, 2599, 1673, 1587, 1357, 1051
03 (5.5%)	Struvite	2362.67, 1459.29 [Channa <i>et al.</i> , 2007]	2858, 1581, 1456, 1052, 871
02 (3.6%)	Cystine	3026, 1618.28, 1485, 846.75 [Marickar <i>et al.</i> , 2009]	3029, 1619, 1479, 843, 538

Table 2. Age wise incidence of kidney stones affectless.

Age in groups	15-25	26-45	46-55	56-72	Total
Male	11	25	06	02	44
Female	02	06	02	01	11
Total	13	31	08	03	55
Female : Male	Ratio				
	1:4				

The morphological examination of specimens depended on the observation of the physical features of the stones, such as shape, weight, texture and colour. Each specimen was examined very carefully and distinguished by its physical appearance. It was observed that the stone samples differed in shape, colour and weight as shown in Fig. 2-4.

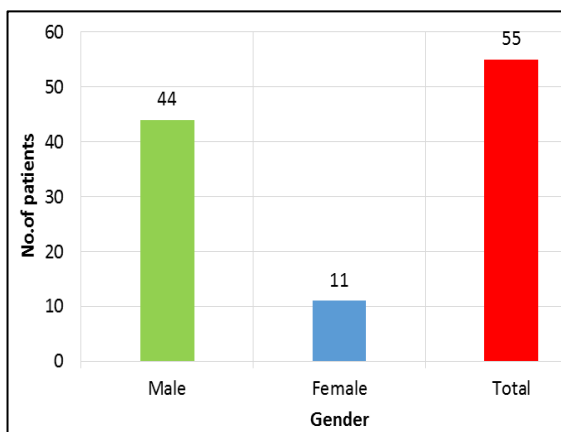


Fig. 1. Gender wise classification of patients.

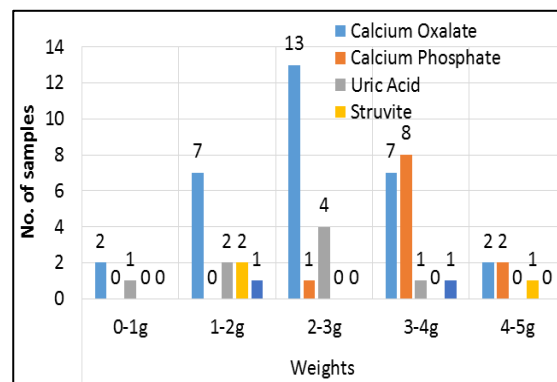


Fig. 2. Weights of the renal calculi samples.

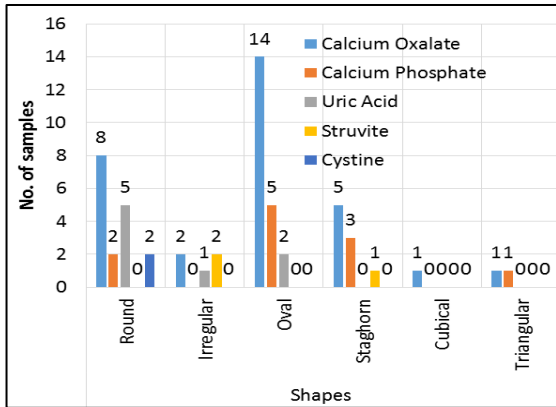


Fig. 3. Shapes of the renal calculi samples.

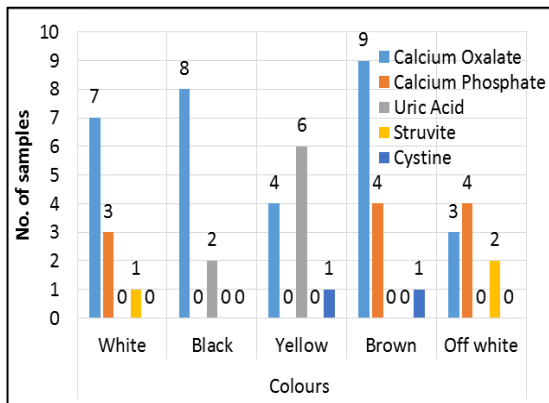


Fig. 4. Colours of the renal calculi samples.

Among all kidney diseases, kidney stone is the major cause of morbidity. It is a multi-factorial disease (Singh and Rai, 2014). The factors such as nutrition, dehydration, heredity, climate and the existence of certain health disorders can increase the risk of developing stones in the kidney (Wróbel & Kuder, 2019). Kidney stone prevalence also varies by age and sex. In the United States, the lifetime prevalence of kidney stones is 12% among men and 7% among women (Pearle, 2001; Pearle *et al.*, 2005). In our study the prevalence among the male and female was found as 80% and 20% respectively and highest prevalence of kidney stones was observed in the age group ranges from 26-45 years. In our current study the kidney stones were also found with different shapes such as round, irregular, oval, triangular etc and the most of the calcium oxalate and calcium phosphate stones were with oval shape. The most of the uric acid and cystine were with round shape and 2/3 struvite stones were found to had irregular shape. The kidney stones also found with different colours. The most of the calcium oxalate, calcium phosphate,

uric acid, struvite and cystine stones were in brown, off white, yellow, and yellow-brown colours respectively. The weight of stones were found in the range 1-5g and the weights of the greater number of calcium oxalate, calcium phosphate, uric acid, struvite and cystine stones were found in the range of 2-3 g, 3-4 g, 2-3 g, 1-2 g and 3-4 g respectively. The findings of our research also showed that the calcium oxalate was the most common 56.4% and also verify the already work done on it in Pakistan that the calcium oxalate stones are more common than others type of kidney stones (Channa *et al.*, 2007; Samad *et al.*, 2017).

Conclusion

This current research work concluded that among all the kidney stones collected and studied, the calcium oxalate was the most common 31(56.4%) and cystine was the lowest 02(3.6%). The kidney stone disease was most frequently observed 31(56.4%) in the age group 26-45 years.

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