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Chemical Analysis of Kidney Stones in Northern Jordan

Barakat M Shabsoug[1*], Adnan Y. AL-Quraishi[2] and Hesham M AL-Mgassgas[2]

1. Department of Chemical Sciences, Faculty of Science and Arts, Jordan University of Science and Technology, **JORDAN**
2. Department of Applied Biological Sciences, Faculty of Science and Arts, Jordan University of Science and Technology, **JORDAN**

Email: bmshabsoug@just.edu.jo

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ABSTRACT

Determination of the chemical constituents of kidney stones aids to establish the etiology of stones disease. The present study was carried out to investigate the composition of kidney stones to provide assistance for appropriate preventive treatment of the patient and to prevent a recurrence of stone formation in northern Jordan population. A total of 50 kidney stone samples were recovered from patients who were admitted to King Abdullah University Hospital (KAUH) and Princess Basma Teaching Hospital, Irbid, Northern Jordan, who were subjected to surgical operation 11 females and 39 males; age range 23 to 85 years during 2013-2014. Gender wise comparison revealed that majority of the stones (78.0%) analyzed were recovered from male patients. Whereas, stones recovered from females were only 22.0%. The composition of all samples was found by the chemical method using BIOLABO analysis kit. The 50 analyzed samples of kidney stones comprised 40.0% calcium oxalate and uric acid mixed stones, 22.0% calcium oxalate stones, 18.0% uric acid stones, 10.0% magnesium ammonium phosphate stones (struvite), 6.0% calcium oxalate-phosphate stones, and 4.0% cystine stones. The relationship between the chemical constituents of stones and both sex and age was established. There were some variations in frequencies of stones compositions according to age. In considering variation between genders, ammonium was higher among female patients whereas cystine stones formed only in males. In conclusion, Calcium oxalate and uric acid mixed stones were the most predominant type in stones of 50 patients.

Keywords: kidney stones, nephrolithiasis, chemical analysis, calcium oxalate.

INTRODUCTION

Nephrolithiasis (also termed renal lithiasis and urolithiasis) is one of common health problems in most countries, which is a pathophsiological condition that occurs when various urine compounds deposit and aggregate forming concentrated structures, these formed structures get bigger as compounds build up around them, which lead to the formation of the kidney stones [1]. This happens specifically on the inner surfaces of the kidneys due to a biological maladjustment where the minerals coalesce and get clumped forming stones that are hard masses like small rocks [2, 3].

Kidney stones usually are made of chemicals such as calcium, magnesium, uric acid, ammonium and other dissolved salts that are found in the urine in their normal concentrations [4, 5]. These salts turn into crystals if they increase in their concentrations and they will get supersaturated and grow to form kidney stones [6]. Kidney stones vary in their anatomical positions such as; ureter, calyx region, renal pelvis and urinary bladder in the urinary system [7]. Kidney stones are also variable in their diameters, they range from small one (in millimeters) like a sand grain which is non obstructing stone so it can pass through the urinary tract without symptoms, to a large one (few centimeters) which cannot drop in the urinary collecting system [8]. Usually, they cause injuries at their sites and block the uretero-pelvic junction in particular, if they are rough, causing classic rhythmic spasms of pain and dysuria as they move [9, 10]. Kidney stones pain can be described as one of the worst pains that afflict humans [11].

The incidence and recurrence of nephrolithiasis have markedly increased in the last decades across the world based on the recent documented epidemiological studies [12]. Up to 5% of the general population is affected by kidney stones, with the lifetime risk of kidney stones estimated at 8-10% [13]. This increase was reported in both sexes, various age ranges and among different races [14, 15]. The increased frequency rates of kidney stones have not been fully elucidated, but like many problems, forming the stones is due to some of nutritional, genetically, and environmental aspects [16, 17]. Because of this increase in the prevalence of kidney stones disease and the variability in their compositions, extensive investigations on the compositional analysis of kidney stones were studied globally [18]. An understanding of the mechanism of stone formation helps to manage patients better, thereby significantly reducing the morbidity and health care costs associated with urolithiasis. Moreover, diagnosing the type of kidney stones is very important because the effective treatments for each type of stones are specific and vary [19]. Therefore, this research aims to explore the etiopathogenesis of kidney stones disease in northern Jordan region, which could be achieved by determining the chemical nature of kidney stones. These purposes are helpful in adopting which enables preventive treatments to minimize stones formation and their recurrence.

MATERIALS AND METHODS

The main materials for this study were kidney stones surgically recovered from patients who were admitted to King Abdullah University Hospital and Princess Basma Teaching Hospital, Irbid, Jordan. A total of 50 kidney stones were collected randomly from patients (uroliths) in northern Jordan who had undergone operative treatment for their calculi in the urology, surgery operation department of King Abdullah University Hospital (KAUH) and Princess Basma Teaching Hospital (PBTH) in Irbid, Jordan. These Samples were surgically removed either ureteroscopically (n=35) or by percutaneous nephrolithotomy (PCNL) (n=15) over a period of 16 months (2013-2014) and were brought to the laboratory in a sterile containers. Patients with kidney stones included in this study were in the age range between 23-85 years in both sexes. Questionnaires covering the information pertaining to patients' sex, age, weight, geographic location, type of diet, water source and the intake of coffee and tea were filed. Also, complete hospital records and health status of patients were documented and analyzed in this study.

Prior to chemical tests, stones were washed carefully with deionized water to eliminate any blood and other residuals, then dried completely at 100°C for 2 hours and weighed. The diameter of each stone was measured (one- dimensional measures). Then the samples were crushed and grinded using clean pestle and mortar to obtain fine ground powder. The powdered stones were weighed, and then incubated at 70°C overnight then analyzed chemically. Chemical method was conducted based on protocols described by Beeler [20]. The stones were analyzed by BIOLABO analysis kit, purchased from Maizy, France. This kit includes reagents for colorimetric (qualitative) determination of main individual inorganic mineral components of urinary stones (Calcium, Ammonium, Phosphate, Magnesium and Carbonate) and organic components (Cystine, Oxalate and Uric Acid) of kidney stones (powdered form). The chemical tests were performed with some mild modifications which were related to optimization between the concentrations of chemical reagents were used in the analysis kit and powdered samples. Reasons for these modifications were due to small quantities of many stone samples. The analysis kit consists of the addition of chemical

reagents labeled R1 to R10 drop wise to the finely pulverized sample and placed into a vessel with 5mL of distilled water. Results were obtained based on changing of dissolved sample color into certain colors or production of effervescence in different chemical tests.

RESULTS AND DISCUSSION

The patients' age ranged from 23 to 85 years. Average age was 51.72 years with S.D of 12.44. The patients included 39 men (78.0%), average age 50.18 years, ranging from 23 to 85 years, and 11 women (22.0%), average age 57.18 years with 33-69 years range. This indicates that men are more likely to develop kidney stones than women by an incidence ratio of 3.54:1[21]. In addition, the average age of affected individuals (51.76 years) was higher than the age averages of previous studies.

In addition to that, female patients represented higher average age than male patients. In male patients, the incidence peaked at age group 51-60 years and then declined. On the other hand women had a peak incidence at age group 61-70 years, which is higher than in men. There were no female patients either 21-30 years age group or above 70 years age group, which indicated a limited age distribution among females, whereas male patients were found in all age groups from 23 to 85 years. This gives an indication that males may form kidney stones in different ages, and this could illustrate that males have a higher incidence percentage than females. Among males, only 4% of kidney stones were found above age of 70 years while only 6% of patients found in 21-30 age groups.

Out of 50 kidney stones patients, 80% were living in nearby villages whereas 20% of patients were living in Irbid city. Their mean weight was 91.6 kg with the range from 81to 98 kg. In addition, small percentages of some common diseases were found in patients like diabetes (8%) and hypertension (10%). This reinforces the notion that nephrolithiasis is a reflection of systemic disease an increased incidence of stone disease is seen in patients with diabetes mellitus, as well as other common systemic conditions, such as hypertension [22, 23].

For each sample, the appearance of kidney stones was observed. The majority of kidney stones have been unique, of different diameters. The diameters of studied stones range from 4 to 13 mm. Colors of kidney stones ranged from yellowish white to dark gray. Kidney stones were different in color, shape, and in their diameters. Chemical tests revealed the presence of mixed stones with highest percent of calcium oxalate with uric acid (Figure 1).

Table 1 shows the percentage values of major constituents for the chemical analysis. As clearly shown, the major detected components were calcium, oxalate and uric acid. Carbonate was not found in all samples. Cystine stones were formed only in males. Uric acid also found in males with higher frequency than in females. In contrast, the struvite stones were more likely to occur in females than in males while the sex of patients showed a difference as far as the composition of kidney stones is concerned for calcium and oxalate.

Figure 1: A dark gray stag horn kidney stone.

Table 1: Chemical composition of kidney stones and their valid percentages

Sex	Frequency	Organic constituents			Inorganic constituents			
		Oxalate	Uric acid	Cystine	Calcium	Magnesium	Phosphate	Ammonium
Males	39	30	20	2	35	3	3	1
Females	11	7	4	-	7	7	5	6
Total (%)	50	37(74%)	24(48%)	4%	42(84%)	10(20%)	8(16%)	7(14%)

Furthermore, relationship between kidney stones compositions and age groups was established. Table 2 summarizes the frequency of stone constituents as frequencies according to the age groups. After dividing the patients into 3 groups according to age: young adults, adults, and elderly people, we found some variations in frequencies of stones compositions according to age. Calcium and oxalate were significantly more common among adults and elderly patients than young adults ($p<0.05$). No uric acid was detected in the stones of elderly patients group as well as ammonium, magnesium, cystine and phosphate. Cystine was exclusively detected in the stones of young adult patients. Ammonium, magnesium and phosphate were detected exclusively in stones of adult patients.

Table 3 shows comparison of kidney stones types observed in the present study with other studies done in KSA, Japan, UK and USA. In all these studies kidney stones were analyzed by chemical methods. Our results showed a clear difference represented by the predomination of mixed stones containing calcium oxalate plus uric acid (40%) while this type of stones was not observed in those studies performed in KSA, Japan, UK and USA. However, pure calcium oxalate stones were the most common variety of stones in the mentioned countries. Our results also showed higher pure uric acid stones than those reported in KSA, Japan, UK and USA, and no pure calcium phosphate stones were observed in our study. All these findings showed a wide distribution of the constituents which could be postulated as a result of variations of diets and environments among various world regions. Other variety of stones showed no big differences between our findings and those reported elsewhere. These include struvite stones, uric acid stones and cystine stones.

The chemical examination of kidney stones represents a pathological biomineral analysis which is a crucial step for the etiology of this disease because it provides a better understanding of the genesis of individual stones which is helpful when prescribing a diet or therapy intended to reduce the risk of recurrence and for patient follow-up. In this study samples of kidney stones were retrieved from patients at King Abdullah University Hospital and Princess Basma Teaching Hospital. Kidney stones were removed either ureteroscopically (n = 35), or by percutaneous nephrolithotomy (PCNL) (n = 15) over a period of 16 months (2013-2014).

We were faced with obstacles in getting more detailed information from patients about the history of stone formation, in addition to other obstacles regarding daily intake of calcium (including supplements and salts), oral intake of fluids and others due to personal privacy and other restrictions. Males were more common among the kidney stones formers compared to females by 3.54:1 ratio. This was consistent with previous studies [28,29] .These findings were, also, in close proximity to reports that have been presented by Singh *et al*, showing that there is a clear male predominance in the formation of kidney stones in the human patients population [30]. The reason for this marked difference and sex related factors is still unknown. Despite of differences in diet between both sexes may be a contributing factor to this fact. The other cause may be that the male urinary tract being more complicated than that of the female [31]. The average of body weight of patients (92.6 kg) was consistent with some recent investigations, which suggested that the obesity and overweight can increase the risk factors and contribute to kidney stones

formation by increasing the urinary content of calcium, oxalate, and uric acid [32]. Moreover, persons who are obese are at higher risk of developing the stones than those who are slimmer, such thing was in agreement with recent studies that have assessed the link between the kidney stones formation and persons being overweight [33].

In our study, we found that almost 78% of the patients were ranging between 40 to 70 years of age. This shows that this disease is common in middle aged and older individuals rather than in younger people. Furthermore, the stones that came second , in frequency, to uric acid and calcium oxalate mixed stones were calcium oxalate stones (24%), followed by pure uric acid stones (18%) that have been linked to hyperuricuria and increased levels of acidity (low pH) [34]. These findings were complementary to the most common kidney stones in this study (calcium oxalate and uric acid stones) which support the clear increase in calcium oxalate and uric acid stones incidence in our region. This is might be associated with consumption of large quantities of milk and dairy products which increase calcium levels (hypercalciuria) and consumption habit of large quantities of red meat [35]. This dietary habit was confirmed by personal communication with patients. Those patients are living in the surrounding regions particularly in northern Jordan villages whose lifestyles are sedentary. This correlated with our statistics done on the patients' population in which we found that 80% of patients were villagers. In addition, these results were consistent with the increase in eating fast foods such as snacks that contain high amounts of processed meat, in recent years by people. All these findings are in agreement with some reports, which have described that vegetarians are at lower risk for stone formation in contrast to non-vegetarians (Robertson *et al*., 1982). In addition, some studies have shown a positive association between animal protein intake and stones formation (Curhan *et al*., 1997). Although dietary calcium is the main contributor to high urinary calcium levels, low calcium diets are not widely used clinically because they are not of proven effectiveness in stone prevention [36] and may predispose to reduced bone mineral content [37]. However, increasing fluid intake is an acceptable method for reducing the recurrence of stones [38]. In addition to that, the majority of patients drink tea daily and this could contribute to increase the risk of stones formation [39]. Other stone types occurred with the expected comparative frequency reported in the literature. The slight decrease in urinary infections stones (struvite) indicates an improvement in hygiene conditions and/or the treatment of urinary tract infections. Males who have the genetic disorder (Cystinuria) are more likely to form cystine stones. Finally, scientific and technological progress led to the spread of amenities and transport thereby reduced the physical activity in recent years, which led to an increase in the proportion of obesity, kidney stones, and other related diseases [40].

Table 2: The relationship between age groups and constituents of kidney stones assessed by chemical analysis.

Age group / Composition	Young adults (21- 40 years) Frequency (%)	Adults (41-70 years) Frequency (%)	Elderly patients (>70years) Frequency (%)
Calcium	44.4	89	100
Oxalate	44.4	89	100
Uric acid	77.7	61.15	-
Ammonium	-	100	-
Magnesium	-	100	-
Phosphate	-	100	-
Cystine	100	-	-

Table 3: Comparison of our results with other countries.

The type of kidney stone	Our study	Japan[24]	USA[25]	UK[26]	KSA[27]
CaOX	22	81.6	58.8	39	72%
Uric Acid+ CaOX	40	-	-	-	-
Struvite [pure]	10	3.7	9.3	15	4%
Calcium Phosphate	-	5.1	8.9	13	-
Cystine	4	-	0.7	3	4%
CaOX + CaP	6	-	11.4	14	-
Uric Acid	18	9.6	10	8	28%

APPLICATIONS

The documented data from patients in the study area of northern Jordan is useful to know the reasons for kidney stones formation in patients, a strong dietary contribution to the development of kidney stones, probably related to the high level of meat consumption. In addition to that, tea is a widely consumed traditional beverage in Jordan, which may contribute to oxalate stones formation.

CONCLUSIONS

Kidney stones can be found in mixture of different chemical constituents as it was observed in our study. This reflects different etiologies in our region, particularly those mixed ones that contain calcium oxalate and uric acid which were the most predominant chemical compositions in stones of 50 patients. The documented data from patients in the study area of northern Jordan showed a strong dietary contribution to the development of kidney stones, probably related to the high level of meat consumption. In addition to that, tea is a widely consumed traditional beverage in Jordan, which may contribute to oxalate stones formation. Moreover, the age at which kidney stones develop varies. Kidney stones are more likely to occur in adults (between ages 41 and 70 years) with the most likely component to be calcium, in combination with oxalate and uric acid. The incidence in men is much higher than in women. We found some differences in kidney stones compositions compared to other countries (Table 3).

This suggests that further studies on this subject are needed considering other regions in Jordan to get more information about the prevalence of certain chemical constituents, its type, and its correlation with environmental, dietary and genetic factors, which may lead to better diagnostic information of this disease and more comprehensive studies regarding this problem.

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AUTHORS' ADDRESSES

1. **Barakat M Shabsoug**
Department of Chemical Sciences, Faculty of Science and Arts,
Jordan University of Science and Technology,
Email: bmshabsoug@just.edu.jo, Mobile (962-777836409)
P.O.Box: (3030) Irbid 22110 Jordan
2. **Adnan Y. AL-Quraishi**
Department of Applied Biological Sciences, Faculty of Science and Arts,
Jordan University of Science and Technology
Email: ayquraishi@just.edu.jo, Mobile (962-799013510)
P.O.Box: (3030) Irbid 22110 Jordan
3. **Hesham M AL-Mgassgas**
Department of Applied Biological Sciences, Faculty of Science and Arts,
Jordan University of Science and Technology
Email: heshammgassgas@gmail.com, Mobile (962-788167210)
P.O.Box: (3030) Irbid 22110 Jordan