Chemical Analysis Study of Kidney Stones in Iraqi Community

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Abstract

The urinary tract system is one of the most important system in human body to control many functions and get any wastes out of body, many diseases may affect this function and prevents it to work effectively. One of this disease is nephrolithiasis at which salt forming stone are settled in renal system affecting its work. The qualitative biochemical analysis of compositions of each stone was done for carbonate, calcium, magnesium, phosphate, oxalate, uric acid, and cysteine, using stone powder derived from the stones. These results found that Calcium oxalate stones were more likely to be detected in patients with upper urinary tract stones ($p < 0.001$). It was observed in 5.3% of cases which was apparently lower than previous data in nationwide urinary stone composition analysis. the most common type of stone was calcium oxalate (84.1%); others were calcium oxalate phosphate (8.0%), ammonia (3.4%), calcium uric acid (2.3%) and calcium carbonate (1.1%). A defect in ammonium excretion would account for the undue urinary acidity; then, increased urine acidity promotes uric acid super saturation. The amount of urinary oxalate excretion is a significant factor in the development of CaOx stones. Aim of study to know how to prevent stone recurrence and formation requiring better knowing mechanisms contributed in this stone formation.

Key Words: Kidney Stones, Iraqi Community, Urinary Tract Infection (UTI).

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Introduction

Kidney stone disease, or nephrolithiasis, is one of the most prevalent urologic pathologies. The disease has existed in parallel with civilization: it is referred to in the Hippocratic Oath, and stones have even been discovered in ancient Egyptian mummies (Kaitlin., 2020). Renal stone formation progresses in successive steps, Nucleation: this regards the phase change of dissolved salts into a solid. It is dependent on the degree of saturation of urine in one solvent (Paliouras *et al*., 2012). growth or gathering of these crystals to a size so that they can interact with some intra-renal structure(s), confinement of these crystals inside the kidney or renal collecting system succeded by further aggregation and/ or secondary nucleation ultimately forming the clinical stone (Ratkalkar *et al*., 2011). Apoptosis at the level of renal tubular cells may lead to stone formation through cellular demise and postapoptotic necrosis which could promote calcium crystal aggregation and growth (Alelign and Petros., 2018). Kidney stones have been associated with an increased risk of chronic kidney diseases, end-stage renal failure, cardiovascular diseases, diabetes, and hypertension (Sigurjonsdottir et al., 2015 and Rule et al., 2010). Uric acid stones may be associated with abnormalities in protein or excessive protein intake and uric acid metabolism but form frequently in the absence of either, as the supersaturation of uric acid rapidly increases in low urine pH (Mehta and Goldfarb., 2012).

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Urinary tract infection (UTI) is very common in patients with kidney stones, which makes the treatment of urolithiasis difficult, even dangerous (Yongzhi et al.. 2018). Calcium stones are the most predominant renal stones which comprises about 80% of total urinary calculi. The calcium stones proportion is divided to pure calcium oxalate (CaOx) (half of total stones), calcium phosphate (CaP, apatite) (only 5%), and more than 45% contributes to mixture of both. Brushite (also called, calcium hydrogen phosphate) is the main constituent and hydroxyapatite. CaOx is found as majority of renal stones and it is present in form of monohydrate, and dihydrate or as combination of mono and di hydrates which are accounting for more than 60%. The second type is called struvite stones which can occur to the extent of one tenth of total kidney stones and have been also referred to be as infection-caused stones and also triple phosphate renal stones. It is frequently happening among patients mainly infected with chronic UTIs that produce enzyme urease which is necessary to cleave the urea metabolite to ammonia and carbon dioxide to make the urine alkaline more and elevates pH (typically more than 7). Uric acid or urate stones accounts for 3–10% of all types of stone. It is caused from diets which are high in purines content especially that are containing animal proteins such as fish and red meat, leads to hyperuricosuria, and repelling low urine volume marked with low urinary pH (< 5.05) that exaggerates uric acid stone forming. Cystine stones comprise less than 2% of all stone types. It is corelated with genetic disease with the transporting of amino acid and cystine.

The objective of this study was to determine the relationship for UTI in patients with urolithiasis.

Materials and Methods

Human Studies

Patients and Sample Collection

Preparation and Processing of Urinary Stone

After the surgical removal, the stone sample was washed several times with sterile water and each stone was then crushed into powder by sterilized mortar and pestle.

3. Analysis of Chemical Compositions of Stones

The qualitative biochemical analysis of compositions of each stone was done for carbonate, calcium, magnesium, phosphate, oxalate, uric acid, and cysteine, using stone powder derived from the second part of stone sections (as aforementioned) that was left after bacterial culture.

1. Procedure

Preliminary procedure phase:

1. **Carbonate dermination** after an examination of the stone to record shape, colour, smoothness, size ., etc crush the urinary stone in the mortar and reduce to powder.

Put approximately 15 mg (or two level spoonful using the blue spatula) of the powdered stone to be analysed in the supplied petri dish . we add 5 drops of reagent 1: a formation bubble / foam indicates the presence of carbonate. We transfer in the 50 mL test-tube using distilled water, completed the volume up to 50 mL and mix well. We withdraw 5 mL of the solution for each parameter (1mL of magnesium only) to be determined and carry out the tests in the supplied 10 mL test tubes.

2. Calcium Determination

Calcium is determined by means of a complexometric titration with calconcarboxilic acid as an indicator.

Procedure: we added 4 drops of reagent 2 and 3 drops of reagent 3 to the sample tube. Mix well. Possibly maintaining a continuous shaking (also manually) then, we added drop after drop, reagent 4 until the colour change from red to blue, count in the same moment the numbers of added drops: the percentage of calcium in the stone is calculated multiplying the number of drops per 5.

Oxalate Determination

Oxalate decoulors the mixture of iron and sulfosalicyclic acid.

Procedure

We added to the sample tube, respecting the following sequence, 2 drops of reagent 5, 3 drops of reagent 6 and 3 drops of reagent 7.

We are let the solution rest for 2 minutes and then compare the colour with the relative colour scale, identifying the relative oxalate percentage.

Ammonium Determination

The ammonium ion forms yellow-brown solutions together with the nessler reagent.

Procedure

We added to the sample tube, respecting the following sequence, 3 drops of reagent 8 and 3 drops of reagent 2 and then compare the colour with the relative colour scale, identifying the relative ammonium percentage.

Phosphate Determination

The phosphomolybdic acid formed in the reaction is redused to molybdenum blue.

Procedure

we added to the sample tube , respecting the following sequence, 5 drops of reagent 9 and 5 drops of reagent 10. We let the solution rest 5 minutes and then compare the colour with the relative colour scale, identifying the relative Phosphate percentage.

Magnesium Determination

Magnesium react with the chemical components and forms a red complex.

Procedure

in a glass test tube we added 1 mL of the sample and 4 mL of distilled water. Shake and then add, respecting the following sequence, 1 drops of reagent 11 and 5 drops of reagent 12. We let the solution rest 1 minute and then compare the colour with the relative colour scale, identifying the relative magnesium percentage.

Uric Acid Determination

Uric acid reduces the phosphomolybdic acid to molybdenum blue.

Procedure

We added 3 drops of reagent 13 to the sample test tube. We shake and let the solution rest 2 minutes, then added 4 drops of reagent 11. Then compare the colour, as quickly as possible, with the relative colour scale, identifying the relative uric acid. The comparation was made promptly because the obtained colour is unstable and tends to change to blue.

Cysteine Determination

Cysteine, in prescence of sodium sulphate, is redused to cysteine, which together with nitroprusside, forms a red colour.

Procedure

We added 10 drops of reagent 14 and 450 mg (or 2 level spoonfuls using of the white spatula) of reagent 15 to the sample test tube. stir until completely dissolved. Wait 1 minute and then added 450 mg of reagent 16. stir until completely dissolved. After 30 seconds compare the colour with the relative colour scale, identifying the relative cysteine percentage.

Result and Discussion

After stone remover by surgical and lazier technique accurate analysis of urinary stone composition is the most crucial laboratory diagnosis procedure for the treatment and prevention in the stone forming patients. Compositional stone analysis should, therefore, be an integral part of the metabolic evaluation of patients with urolithiasis (Siener *et al*., 2016). Incorrect analysis or failure to identify a stone substance may result in inadequate therapy.

A total of 31 patients have stone in urine for both sexes, 12(38.7%) male and 19(61.2%) female with age range from (25-66 years for males) and (23-65 years for females). Out of 31 patient samples have stone in urine, 12(38.7%) stone had one component, 7(22.6%) stone consist of two components and 12(38.7%) consist of three or more components, the results were shown in Table (3-2).

Table (3-2). Distribution of stones composed of single or multiple components

No.	Stone with single components	No.	Stone with two components	No.	Stone with three components	No.
1.	COM	4	COM/COD	2	COM/COD/uric acid	3
2.	Uric acid	2	COM/uric acid	2	Cysteine/COM/COD	4
3.	COD	2	ACP/COM	1	ACP/uric acid/COD	2
4.	Cysteine	1	Cysteine/COM	1	COM/COD/ACP	3
5.	ACP	3	COD/uric acid	1		
Total (31)		12		7		12

COM: calcium oxalate monohydrate, COD: calcium oxalate dehydrate ACP: amorphous calcium phosphate

However, Calcium were found in 30(96.7%) stone, uric acid in 29(93.5%) stone Magnesium in 26(83.8%) stone, oxalate in 12(38.7%) stone, cysteine in 2(6.6%) stone, ammonium in 3(9.6%) stone and phosphate in 1(3.2%) stone as shown in Table (3-3).

Table (3-3). Distribution of main urinary stone composition (No. 31)

No.	urinary stone composition	No. of patients	mean±SD	P value
1.	Calcium	30(96.7%)	15.2±8.9	0.001
2.	Uric acid	29(93.5%)	21.12±17.6	0.001
3.	Magnesium	26(83.8%)	8.2±6.27	0.001
4.	Oxalate	12(38.7%)	8.06±13.05	0.001
5.	Cysteine	2(6.6%)	2.58±11.06	0.001
6.	Ammonium	3(9.6%)	0.35±1.7	0.001
7.	Phosphate	1(3.2%)	0.64±2.10	0.001

These results were agreement with results obtain by Wang *et al*., (2019) who found that, Calcium oxalate stones were more likely to be detected in patients with upper urinary tract stones ($p < 0.001$), while Haas *et al*., (2020) found that, infection stones were more likely to be detected in patients with stones in urine ($p = 0.001$). Zhang *et al*., (2021) found that, the prevalence of CaOx was (78.37%) of stones in urine.

Several studies have demonstrated that, dietary oxalate notably leads to urinary oxalate excretion (Holmes *et al*., 2016; Crivelli *et al*., 2021). The amount of urinary oxalate excretion is a significant factor in the development of CaOx stones.

Patients with calcium oxalate stones benefit from decreased oxalates in their daily diet (Prezioso *et al*., 2015).

Zhang *et al*., (2021) found that, ammonium stones are second only to calcium-containing stones in prevalence, it was observed in 5.3% of cases which was apparently lower than previous data in nationwide urinary stone composition analysis. A defect in ammonium excretion would account for the undue urinary acidity; then, increased urine acidity promotes uric acid super saturation. Urinary pH is the crucial determinant of uric acid crystallization (Ma *et al*., 2019).

Shah *et al*., (2020) found that, the striking new trends of increased incidence of stone formation might be due to associated risk factors such as increasing obesity, dietary changes, and change in fluid intake patterns. It was found that, stones isolated from patients were subjected to qualitative biochemical analysis, the most common type of stone was calcium oxalate (84.1%); others were calcium oxalate phosphate (8.0%), ammonia (3.4%), calcium uric acid (2.3%) and calcium carbonate (1.1%), Khattak *et al*., (2021) found that, the infective stone accounted for (71.9%) calcium oxalate stones determined.

Kidney stones and ureteral stones were more commonly identified as calcium oxalate stones, and bladder stones and urethra stones were more generally confirmed as infection stones, which indirectly suggested that the causes of upper and lower urinary tract stones are different (Kittanamongkolchai *et al*., 2018).

Knowledge of the composition of urinary stone is an important, and sometimes the only, indication of the disorder, therefore it must be known (Bianchi *et al*., 2020). Stone components may be mineral, organic, or both. The same chemical components may crystallize in different forms; for instance, calcium oxalate may be present in the form of calcium oxalate monohydrate (COM), dihydrate (COD), or trihydrate (COT), therefore proper stone analysis has to identify not only the molecular species, but also the crystalline form within the chemical constituents (Šafranko *et al*., 2021).

Urinary oxalate is a product of both exogenous dietary consumption of the compound and endogenous production as a terminal product of metabolism. It is estimated that 20-50 % of urinary oxalate results from dietary consumption (Dobrek, 2020).

Calcium stones are the most common type of kidney stones, and can be either calcium oxalate or calcium phosphate. As mentioned, good hydration is important to prevent calcium stones. It may be surprising, but results of a randomized clinical trial show that, people with calcium kidney stones should not cut back on dietary calcium. In fact, they should consume the recommended daily allowance of calcium (1,000 mg/day for women younger than 50 years old and men younger than 70, and 1,200 mg/day for women over 50 and men over 70) (Meyers & Naicker, 2021).

Calcium phosphate stones are less common than calcium oxalate stones. Causes include hyperparathyroidism (when the body produces too much parathyroid hormone), renal tubular acidosis (a kidney condition that causes a buildup of acid in the body), and urinary tract infections. It is important to understand if one of these conditions

is behind the formation of calcium phosphate stones (Singh *et al*., 2021).

Good hydration can help prevent recurrence of calcium stones. In addition, thiazide diuretics such as hydrochlorothiazide can help the kidney absorb more calcium, leaving less of it in the urine where it can form stones. Potassium citrate is another medication that can bind to calcium and help keep calcium oxalate and calcium phosphate in the urine from forming into stones (Memane *et al.,* 2020).

Most patients with uric acid stones don't have too much uric acid. Instead their urine is too acidic. When that happens, normal levels of uric acid dissolve into the urine where it can crystalize into stones. Adjusting the pH of the urine, most commonly with the medication potassium citrate, reduces the risk of uric acid stone formation and can also help dissolve existing stones. Sodium bicarbonate can also be used to alkalinize the urine. Some people with uric acid stones do produce high amounts of uric acid (Costa-Bauza *et al*., 2018).

Trace elements are essential components of biological structures and are necessary for a multitude of vital functions in the human body. On the other hand, these elements can be toxic at concentrations exceeding those necessary for their biological functions (Zoroddu *et al*., 2019).

The precise role of trace constituents in the pathogenesis of renal calculus is still unclear and under debate, but researchers are increasingly focusing on the role of trace elements in lithogenesis (Chandrajith *et al*., 2019). Trace elements such as zinc (Zn), copper (Cu), nickel (Ni), aluminium (Al), strontium (Sr), cadmium (Cd) and lead (Pb) form poorly soluble salts with phosphate and oxalate ions and therefore play an important role in kidney stone formation. Trace elements also affect the speed of the crystallization process and influence the external morphology of growing crystals (Lindholm-Lehto, 2019).

Aghajari *et al*., (2021), investigated the effects of a number of trace elements on the inhibition of calcium oxalate crystallization, resulting in a great step forward towards elucidating the pathogenesis of urinary stones.

Alamani *et al*., (2021) analyzed trace metal contents in calcium oxalate and a mixture of calcium oxalate and calcium phosphate and tested the inhibitory effect of each of these trace metals on the crystal growth of calcium oxalate and calcium phosphate. Other study of Han *et al*., (2021) have focused on the effects of trace elements on solubility and crystallization, and the determination of trace element content in humans has attracted increasing attention.

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