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Inspection of some causes of renal calculi in children under the age of 15 years

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Abstract

Children at earlier age are less susceptible to urinary tract infections, but there are some factors that may lead to stone formation. The study was conducted to investigate the most important physiological and bacterial factors affecting the frequency of stone formation in some children in Anbar province. This was done through the use of chemical variables in serum and 24 hours urine (calcium, uric acid, citrate, oxalate, potassium, magnesium, inorganic phosphorus, urea, creatinine, creatinine clearance, total pH and total volume), also detection the role of chemical and bacterial analysis of the stone in the metabolic evaluation of kidney disease. The chemical analysis of the stone showed that the uric acid stone was the highest with (49%), calcium oxalate (27%), calcium phosphate(16%)and the struvite(8%)in children aged (5-15) year , Percentage of children with kidney stones less than 5 years were 20% , calcium oxalate compose 9.6% of the total other stones, the rest of the species were 5% of uric acid struvite 3% and calcium phosphate 3 %. No rare stone were detected. When comparing the group of patients with the control group calcium, uric acid, oxalate, sodium and phosphorus in the 24 hours urine, at a higher level than the control group with a significant statistical difference, while citrate , magnesium, potassium and creatinine in the urine were at the lower level than the control group with a significant statistical difference as well. At the same time, the group of patients has a higher serum creatinine than that of the control group. It also has a lower creatinine clearance value and a significant statistical difference than that of the control group. Understanding the factors involved in urinary the formation of kidney stone in children and the right investigations presenting ,will allow for earlier recognition and assist in the prevention of the recurrence. Bacterial identification showed positive isolates of urease, including *Pseudomonas aeruginosa*, *Proteus mirbilis, Klebsiella spp*., *Staphylococcus aureus*, and other negative tests, which included *E. coli* and *Streptococcus ssp*., and identified of five bacterial species isolated from different types of stones. *E.coli* were the most predominant isolates from the stones samples followed by *Pseudomonas aeruginosa, Proteus mirbilis, Klebsiella spp., Staphylococcus aureus* respectively.

Key words: kidney stone, children, kidney function, bacterial identification

INTRODUCTION

It was previously believed that children at the beginning of life are less susceptible to urinary tract stones in general and kidney stones in particular, and this situation is increasing continuously with changes in lifestyle and changing the nature of food eaten by mothers and children, (1). In certain regions, such as Southeast Asia, the Middle East, India, and Pakistan, calculi are endemic. Calculi are particularly uncommon in children of African descent (2).

Total stones are formed due to the accumulation of crystals of certain chemicals in the urine, especially if the urine is high concentration of these substances. Calcium oxalate is one of the most common types of stone in most industrialized and developing countries, where food quality and some diseases are factors affecting accumulation, and this type of stone is accompanied by severe pain as well as difficulty in treatment in advanced cases (3). Several studies have shown that the lack of intake of sufficient amounts of water and fluids is added to the multiplication of foods whose accidental product is oxalate, such as some fruit, soft drinks, chocolate, pistachio butter, and other types of risk. Other risk factors for renal stones are obesity, insulin resistance, gastrointestinal pathology, living in warmer climates, and certain dietary patterns and medications.(4,5and 6)

Abdominal pain occurs in approximately half of the children severing with urolithiasis, but the classical description of excruciating loin pain associated with the passage of the stone is uncommon. In infants stone symptoms may often be confused with colicky abdominal pain. Microscopic hematuria is present in over 90% of children with stones, and the possibility of urolithiasis should always be considered in children with non-glomerular hematuria.(7) Urinary tract infections are often associated with renal calculi and pyuria may be seen.

Some studies have indicated a relationship between bacterial urinary tract infection and kidney stones. About 70% of the bacteria isolated from the stones were identified through bacterial culture. These studies helped to select appropriate treatment and prevent recurrence of the stones (8).

The bacteria present within the remains of the stones after surgery show high resistance to antibiotics and the high rates of recurrence due to the presence of bacteria in the remains of stones, that make treatments conventional is ineffective and success in controlling the infection becomes more difficult.(9) serious complications that arise from infection of urinary tract infections, which are caused by various bacterial species, especially the Proteus mirabilis bacteria, the formation of bladder and kidney stones, the formation of the biofilm and bacteremia due to the possession of virulence factors and the urease enzyme, which analyzes urea to NH_3 and CO_2 as a final product, thereby increasing the alkaline of urine. (10)

It is worth mentioning that children at the beginning of age are vulnerable to kidney stones if the mother suffers from some health problems associated with pregnancy and the wrong diet and the lack of consumption of sufficient amounts of water and fluids, (11) in pregnant women Renal plasma flow and the glomerular filtration rate both increase by over 50% during pregnancy (12). This leads to increased urinary excretion of calcium, uric acid, sodium, and oxalate, all of which are lithogenic, Calcium tubular reabsorption is also reduced due to suppression of parathyroid hormone (13) so when the mother's intake of nutrients during pregnancy and the large container of calcium, the child be exposed to accumulation of this substance in the body and therefore develop to be stone and because women are more susceptible to infections of the urinary tract because of the synthetic nature of the urinary system, chronic infections may affect the fetus and develop into a larger problem.(14 and 15).

The aim of the study is to identify some of the causes of stone formation in children from the beginning of age to 15 years through a comprehensive assessment of the functions of the kidney and estimate the glomerular filtration rate (GFR) of creatinine, which is the most important indicator of renal function problem. Identification of the components of the stones through the quantitative and qualitative analysis of the extracts taken from patients from different regions of the central and northern regions of Iraq.

MATERIALS AND METHODS

Blood and urine samples were collected from 110 children, including 75 with kidney stones from different regions of AL-Anbar province. The patients were aged from 6 months to 15 years. Patients were diagnosed by specialists in kidney and

urinary tract diseases through clinical diagnosis and symptoms and examinations X-ray and ultrasound. Information on the health status of the patient and the symptoms associated with the disease were collected by the patient or accompanying him, and by using some observations by the treating physician.

For comparison, blood samples were taken from 35 people aged 6 months to 16 years who were healthy and had normal renal function, so considered as control group .

Samples

1. Serum
   Blood samples were collected and the serum was isolated within 5 hours and stored under 70 $\overset{o}{C}$ until tests for kidney function were performed.
2. Urine for chemical variables evaluation
   The urine sample was collected within 24 hours and the patient was guided in a healthy way to collect and preserve the urine in clean, sterile and sealed bottles.
3. Urine for bacterial detection
   Fresh urine collected directly for general urine examination.
4. Stone
   The samples of stone were collected by the patient or his family or by the treating physician during the surgical operations and then placed in plastic packaging clean and well washed and saved and then chemical analysis of stone contents and bacterial analysis was done.

Methods

A. The components were estimated in serum and urine by using spectrophotometric methods and specialized kit for :

(Urea, uric acid, Creatinine, Calcium, Inorganic phosphorus, Potassium and sodium, Citric acid) by BioSystems S.A. kit (Spain).

Creatinine clearance

$$= \frac{\text{mg creatinine/100 ml urine} \times \text{urine volum (ml/24 hrs)}}{\text{mg creatinine/100ml serum} \times 1440} \text{ ml/min}$$

B. pH and total volume of urine

C. Stone Analysis

1 Qualitative analysis of the stone

A qualitative analysis of stone is carried out to detect the presence of carbonate, phosphate, oxalate, uric acid, and cysteine.

The composition of each sample was determined using a stone analysis kit (DiaSys Diagnostic Systems(Germany)). The kit contains all necessary reagents for semi-quantitative measurement of the most important components of kidney stones including calcium, oxalate, magnesium, phosphate, ammonium, uric acid and cystine. Initially a homogenous powder was prepared from each sample and dissolved by adding some pure sulfuric acid and the final volume was adjusted to 50 mL using distilled water. The prepared samples were used for determination of di fferent parameters using the mentioned kit

2. Bacterial analysis of the stone

A. Isolation of bacteria

1.Stone samples

Part of each type of stones were culturing in brain heart infusion broth medium.

2- Urine samples

The urine samples were taken directly and plated on different culture media for the purpose of bacterial identification and biochemical tests.

Each of samples were prepared by plated on 7% sheep blood agar, MacConkey agar, Nutrient agar, urea agar, Eosine Methylene blue, pepton water medium, Simmon citrate agar, VP-MR medium and Triple sugar iron agar, these media were prepared according to manufacturer's instructions. All the plates incubated aerobic conditions at 37 C for 24 h.

B. Identification of bacteria

Identification of bacteria depended on, morphological and biochemical characters. To study the cultural characteristics, observed of discrete the colonies on the agar surface: the shape, size, cosistenty, color and pigment production. The cellular morphology of isolates were observed by staining the isolates with gram stain and examined microscopically. The biochemical tests include: catalase, oxidase, (triple sugar iron), coagulase test, IMVC test (indol production, methyl red, vogas-proskauer and citrate utilization), TSI, urease production, gelatin liquefaction, hemolysis test on sheep blood agar and different carbohydrates utilization. The methods analysis for identification of bacteria were done depended on (16).

RESULTS AND DISCUSSION

The fact that kidney stones is a disease that causes pain and many complex complications, which causes great danger to the life of the patient, especially the patients under study, the children and most of them are not able to explain their suffering to the surrounding, so provide adequate information about the disease and study the causes of the occurrence in children It is easy to develop appropriate treatment for this situation and to rid the injured child of suffering that may grow up with him and lead his life.

The results of kidney function tests in both serum and urine 24 hours showed significant differences in some of them, while there were no differences in the other

Statistical analysis of chemical variables in the serum of patients and control samples (table 2) showed that there were significant differences ($P < 0.01$) in the values of magnesium, inorganic phosphorus, potassium, uric acid, and creatinine in the serum of the patients, compared with the control samples, while the statistical analysis did not show any significant differences ($P < 0.01$) Calcium, sodium and urea have remained within normal values compared to control samples.

Table 1: Chemical variables in the serum of patients and control samples

Variables	Samples	Control	Value
Urea	33.25±7.7	33.25±7.7	-
uric acid	6.02±1.5	4.5±1.36	P< 0.01
Creatinine	1.09 ± 0.4	0.9±0.3	P< 0.01
Calcium	9.231±1.11	9.132±1.65	-
Inorganic phosphorus	4.37±0.24	4.24±1.1	P< 0.01
Sodium	125.007±25.6	134.9±22.4	-
Potassium	36.14±11.8	6.12±6.04	P< 0.01
Magnesium	2.35±0.74	2.92±1.12	P< 0.01

P<0.01 means there were a significant differences in the variables

The results of the statistical analysis of Chemical variables in 24h urine (table 1)showed significant differences ($P < 0.01$) in the values of magnesium, calcium, uric acid, inorganic phosphorus, potassium, sodium, pH, and statistical analysis showed no significant differences In the values of oxalate in patient administration and remained within normal values when compared with control samples.

The most important factors causing the disease in children is the nature of food, where it was noted that most children, especially from the ages of 10 to 15 years, had food contains foods saturated with fats and soft drinks according to information taken from the child himself or his family. The most influential ingredients are some soft drinks containing oxalate, especially cola and chocolate, which is widely consumed by children in that age. (17) show that Sodas contain high levels of phosphoric acid, which has been linked to kidney stones and other renal problems.

Table 2: Chemical variables in the 24 hour urine of patients and control samples.

Variables	Samples	Control	Significance
Urea	31.15±1.7	34.90±10.10	-
uric acid	2.5±1.2	5.02±2.5	P< 0.01
Creatinine	8.02 ±4.7	12.12 ±2.41	P< 0.01
Creatinine clearance	89.3 ±11.3	99.1 ±11.4	P< 0.01
Calcium	132.4±43.8	115.4±89.6	P< 0.01
Inorganic phosphorus	301.89 ±186.66	117.78 ±23.34	P< 0.01
Potassium	3.54±0.7	99.8±72.3	-
Sodium	204.5±200.3	125.12±10.9	
Citric acid	206.07±137.05	490.3±63.12	-
Magnesium	122.78±34.4	176.081±10.15	P< 0.01
Ph	6.125±0.5	6.555±0.69	P< 0.01
Oxalate	112.61±302.1	33.53±6.8	-

P<0.01 means there were a significant differences in the variables

Several studies have indicated that there is a linear relationship between gastrointestinal absorption of oxalate and the amount of calcium intake in food. This is explained by patients following a calcium-restricted diet and at the same time not restricted to oxalate. This is confirmed by a study of the diet of oxalate Calcium (18) showed that reducing or limiting calcium-containing foods would allow for greater absorption of oxalate in the intestine, leading to excessive excretion of oxalate in the bloodstream and causing hypoxaluria.

The citrate play an important role as an organic inhibitor to form stone through its effect in the crystallization process and nucleation to prevent the growth and accumulation of crystals and thus prevent the formation of stone as well as with calcium to be a high-solubility complex and thus reduce the ionic concentration of calcium and saturation of the oxalate crystals and calcium phosphate (19). From this we conclude that the low level of citrate in the samples of 24 hours of patients in this study is one of the reasons and risk factors in the formation and frequency of stone.

Urea and creatinine were measured to test kidney function and their ability to filter ions and salts. Creatinine is the most non-protein nitrogenous protein of urea to reverse kidney function because its concentration in the blood is not affected. Urea is also affected by the amount of protein consumed in food, Have a re-uptake by the kidney and therefore the rise in blood plasma percentage leads to a rise in the rate of generation if the other factors (glomerular filtration) constant (20).

The pH of the blood was found to be lower in the patient samples than in the control group. This difference was significant (P <0.01). This result was consistent with the results of other studies (21 and 22) .These studies showed an increase in the pH values in the samples compared to the samples of patients with urotithisis.

The results of the chemical analysis of the stone showed the presence of different types, shapes and colors of gravel, while the weights of gravel ranged from 10 mg to 153 mg. Figure (1) show the different shapes and colors of kidney stones Figure (2,3) show the percentage of renal stone types in patients according to age.

Percentage of children with kidney stones less than 5 years were 20% . calcium oxalate compose 9.6% of the total other stones, the rest of the species were 5% of uric acid struvite 3% and calcium phosphate 3 %. Most of the patient there mothers were suffering from some problems associated with pregnancy in addition to the intake of nutrients rich in calcium.

For the rest of the ages, the highest percentage was for uric acid, 49%, calcium oxalate 28%, calcium phosphate 16%, and the remaining 8% for struvite (M.A.P.), This is in line with studies that show that kidney stones are increasing in age and are more frequent in teenagers than in children (23) show that increasing age is a risk factor for kidney stones, adolescents are more likely to form stones than younger children. None of the other types of stones, which are rare species, such as cysteine, zanthane and silica, have been shown. Cystine stones are very rare, and happen in people who have a genetic disorder that causes cystine to leak from the kidneys into the urine. According to (24), other types of rare stones like dihydroxyadanine, stones resulting from protease inhibitor drugs and may also occur.

Figure (1) show the different shapes and colors of kidney stones

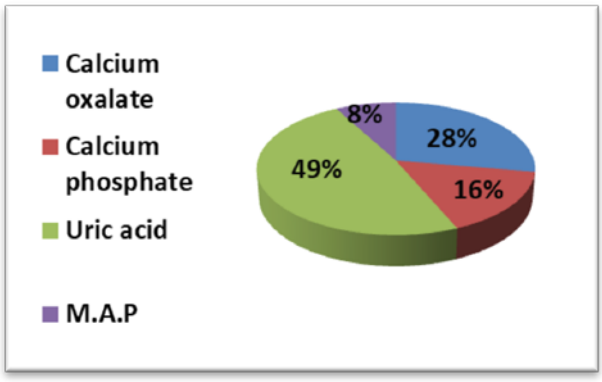

Figure (2) show the percentage of renal stone types in patients aged(6mothe – 5 years)

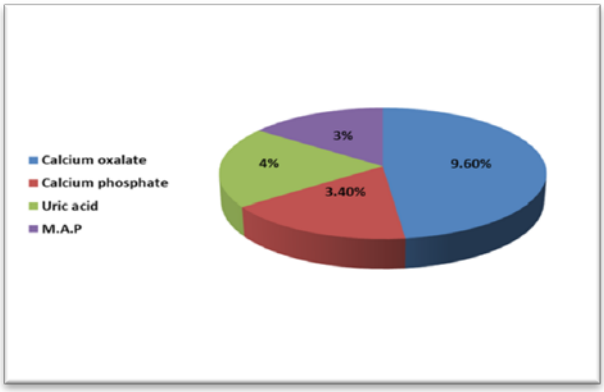

Figure (3) show the percentage of renal stone types in patients aged(5-15)year

The increased intake of food rich in animal proteins is the main cause of the formation of stone, especially uric acid stones, as confirmed in many researches (25, 26 and 27)found that the increased intake of animal proteins, especially red meat is directly associated with the risk of uric acid stones and this explains the high proportion of acid stones uric acid stone in the advanced ages of the study. The reason for the increase in the percentage of uric acid in Anbar province due to the nature of nutrition for the population of this region as the vast majority of them consume animal proteins, which are red meat significantly. This interpretation corresponds to(28)that high consumption of animal proteins directly correlates with the recurrence of kidney stones. (29) found that uric acid is more widespread and common in

developing countries, The end of the last century and this reflects the increase in the consumption of animal protein with food in these countries.

Struvite (M.A.P.) was 3.57% of the total number of pests in this study. This percentage is less than the overall range of infection, which accounts for 5% -10% of the total number of pests as indicated by many studies and research in different countries (30 and 31).

The study showed that 5% of the children under study were suffering from health problems, including diabetes, as well as excessive weight and excessive obesity. This is consistent with what was found to be that the increase in diabetes and some hormonal diseases and excessive obesity accompanied by a possible child kidney stones (32 and 33).

The results showed positive isolates of urease, including *Pseudomonas aeruginosa, Proteus mirbilis, Klebsiella spp., Staphylococcus aureus*, and other negative tests, which included *E. coli* and *Streptococcus ssp.*, and identified of five bacterial species isolated from different types of stones. The results observed that *E.coli* bacteria were the most predominant isolates from the stones samples followed by *Pseudomonas aeruginosa, Proteus mirbilis, Klebsiella spp., Staphylococcus aureus* respectively. while eight of samples were negative bacterial growth.

UTI samples have been diagnosed with 6 bacterial strains, the same species isolated from the stones as well as bacteria *Streptococcus ssp*.

It was also the largest percentage in the isolation for *E.coli* bacteria, Where the results showed that the isolation rate was 37% of the total other isolates and the lowest proportion of *Streptococcus ssp.* bacteria by 7%. Table (3) and figure (4)

Table (3): Bacterial species isolated from Stones and UTI samples and its percentage

Bacteria	Stones		UTI		Total	(%)
	No.	(%)	No.	(%)		
Escherichia coli	32	43	28	37	60	40
Pseudomonas aeruginosa	19	25	15	20	34	23
Proteus mirbilis	7	9	11	15	18	12
Klebsiella spp.	5	7	9	12	14	9.3
Staphylococcus aureus	4	5	7	9	11	7.3
Streptococcus ssp.	-	-	5	7	5	3.4
No growth	8	11	-	-	8	5
Total	75	100	75	100	150	100

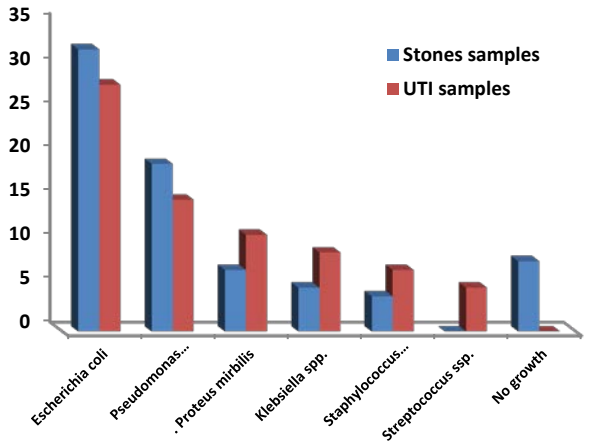

Figure (4): Comparison between the numbers of isolated bacteria from the stones and UTI

The results of the study were agreed with the results of the study (34), which showed that *E. coli* was the most common among the species of isolated bacteria by 30.76%, followed by bacteria *Pseudomonas aeruginosa* with 21.22% and then bacteria *Proteus mirbilis* by 15.38%. while *Staphylococcus aureus* was the lowest types of isolated bacteria And by 1.92% .

Also our results agreed with (35) *E. coli* was the predominant isolate isolated from the urine specimen followed by *Pseudomonas aeruginosa, Klebsiella pneumoniae* but the *Proteus mirbilis* was the lest isolated.

The results differed with (36) which showed that the bacteria *Pseudomonas aeruginosa* were the most proportion of most types of stones diagnosed while *Proteus mirbilis* isolated in ratio 8.33%.

The researcher (37) isolated bacteria *Proteus mirbilis* with 92% of the cases of urinary tract infection and 22% of these bacteria from children under the age of 13.

Seventy percent of the stone forming bacteria can be identified through urine cultures. These studies are useful in selecting antimicrobial agents for preventing stone regrowth or stone recurrences or sterilization of stones . Studies with larger samples may be needed to determine the relationship between species of microorganisms and composition of stones.(8)

Among the bacterial species identified it is *Proteus mirbilis* bacteria , which is the reason for the formation of stones that have the ability to produce urease and its resistance to the high concentrations of urea as well as its ability to grow in alkaline medium and analysis of urea to get energy and nitrogen. (38)

Also, many Gram negative bacteria within the components of the stones show the effectiveness of the urease enzyme and the viscous layer produced by the bacteria and the formation at the beginning of the emergence of the components of the stones, which are less pronounced in mature stones due to increased mucosal proteins in the host. (39)

The presence of *E. coli* and non-urease-producing bacteria in stone samples is due to the development of postoperative infection to remove stones, especially patients with phosphate-containing calculi (40). Also the presence of bacteria in combination with other bacterial species is a cause of urinary tract infection and the formation of stones (41).

Through inquiry and collection of information on each patient, it was found that children under the age of two years had their mothers suffering from some of the accompanying disorders of pregnancy such as anemia, nutrient deficiency, urinary tract infection and the treatment of certain types of treatments and nutrients containing calcium, which causes accumulation and thus transmission to the fetus By the placenta or by natural breastfeeding and thus evolve to be gravel inside the child's kidney.

Prevention Measures to prevent kidney stones include dietary modifications, nutritional supplements, and medications, depending on the specific type of kidney stone and urine characteristics (42 and 23).

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