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College of Education for Pure Science
Department of Chemistry

Determination of the Chemical Content of kidney Stones for Patients in Karbala Governorate

A Thesis

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By

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Dedication

To all the bright stars that shine, guide me to success,

to my family …. the backbone of this life

(My father, Mother, Brothers, and Sisters),

I dedicate this work for all of them, and I dedicate this work to people who value meaning and creativity. Through the sincerest feelings and the best words emanating from the heart,

I extend my thanks and gratitude to those who were the reason for the success of my career and those who stood with me in the most demanding and motivated circumstances.

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Summary

This study aims the determination of the chemical content of kidney stones for kidney patients in Karbala Governorate and the identification of the places most at risk of contracting the disease, as well as the races and ages most at risk of contracting the disease. Urolithiasis is the formation of stones in the kidney, bladder, and/or urethra is increasingly common, with a rate of approximately 12% worldwide, and it is associated with an enhanced risk of end-stage renal disease. The most common form of kidney stone is calcium oxalate (Ca Ox) on the renal papillary surface.

Stone formation involves a complex process that results from a number of physicochemical occurrences namely, supersaturation, nucleation, growth, aggregation, and stone retention within tubular cells. Additionally, cellular injury furthers the retention of particles on the renal papillary surface.

Generally, varied stone compositions are found in different regions, but the exact data are lacking because of paucity of large population, geography, lifestyle, and dietary habits. This study aims to determine the chemical composition of kidney stones.

The study was performed using various analytical methods such as, atomic absorption, and spectroscopy infrared radiation. Samples were collected for patients with kidney stones in hospitals of Karbala Governorate after surgery for eradication in the Department of Urology.

The age range were divided into three age groups (13-31) years, (32-49) years, and (52<) years. The kidney stone were more common in patients ages (52<) years. The number of males was 20 (57%) and the number of

females was 15(43%). The distribution of stones in Karbala governorate was calcium stones (80%), oxalate stones (57%), pure uric acid stones (34%), a mixture of calcium phosphate stones with calcium oxalate monohydrate stones (26%), and a mixture of calcium oxalate monohydrate stones with struvite stones (11%), while Cysteine was at a percentage of (3%).

The results showed a high percentage of calcium and oxalate stones in the Karbala governorate, where they were more in females than males. This can be due to the nutritional factor or dairy products and not drinking enough water may play a major role in the stone's formation

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Symbols	Abbreviations
SPP	Streptococcus pyogenes
GFR	Glumerular filtration rate
Coax	Calcium oxalate
Caph	Calcium phosphate
COD	Calcium oxalate Dehydrate
COM	Calcium oxalate monohydrate
COT	Calcium oxalate trihydrate
CT	Computed tomography
OCPP	Octa-Calcium Phosphate Pent hydrate
SEM	Scanning electron microscopic
TGA	Thermo gravimetric analysis
UA	Uric Acid
UTI	Urinary tract infection
XRD	X-ray diffraction

Chapter One

INTRODUCTION

1-1 Kidney

Humans are naturally born with two kidneys, located on either side of the vertebral column on the dorsal side below the rib cage. Stretches from the twelfth chest spine to the third cotton paragraph, one on the right side and the other on the left side, which is higher than the right with the liver on the right side [1]

They are two bean-like organs with a typical adult length of 12-10cm Display 7-5 cm (3-2 inches); 3cm (1in) thickness; they weigh 150-135 g (5-4.5 oz) [2] The kidney contains 105 A million rental units. They serve as filters for the disposal of organic waste from blood and the elimination of nitrogen waste, especially urea in urine [3]

The kidneys are central to maintaining balance [4]. Through remarkable sensory mechanisms [5], they regulate blood pressure, water [6], sodium [7], potassium [8], acidity bone minerals, and hemoglobin. However, its primary function is to release waste from urine metabolism. [9]

About 22% of the cardiac output goes to the kidneys, and about 20% of plasma is filtered, producing about 170 liters of glomerular filtrate per day. Ninety-nine percent of this is reabsorbed as it flows along the nephrons, so only about 1.5 liters of urine are produced daily [3]

The primary function of the kidneys is to maintain a constant composition of blood and tissue fluid and to achieve the kidneys extract the extractive substances from the blood and put them out into the urine, i.e., purify the blood from impurities; Some of these substances are useful but exceed the need of the body such as creatinine and there is a cross-body type such as caloric nitrogen waste and uric acid. Blood also disposes of excess water and thus maintains a constant content. Water in the blood i.e., adjust water levels, minerals (calcium and phosphate), and salts (sodium, potassium, and chlorine) from. It is also maintaining essential blood ingredients that the body excise them. [10]

1-2 kidney stones

Kidney stones are a buildup of a complex and precipitated mineral component from a highly saturated solution. Kidney stones can cause blockages in the urinary tract, leading to stagnant urine and creating a suitable environment for bacteria that cause urinary tract infections. This blockage may also pave the way for the spread of infection to the upper urinary tract.[11]

Urine contains many minerals and soluble salts. urine has high levels of these minerals and salts. Kidney stones can start small, but they can grow larger, even filling the hollow internal structures of the kidney. Some stones remain in the kidney and do not cause any problems. Sometimes, kidney stones can move down the ureter, the tube between the kidney and the bladder. A stone that leaves the kidney and gets stuck in the ureter, it is called a ureteral stone [12]

Over the past few decades, the incidence of urinary stones has been increasing. Generally, nearly 10% of the population in Western countries is affected. Significant changes in dietary habits, including high protein and salt intake, and more recently, high consumption of fructose-rich soft drinks is one of the main reasons for the increased incidence of calcium oxalate kidney stones which is now the most common type of stone. However, many other factors may be involved in stone formation. More than 100 chemical components have been identified in urinary stones, and more than 100 different causative agents may participate in stone formation [13]. Among the analytical methods for determining the components of stones, chemical, and physical methods can be used. However, despite their low cost, chemical methods are often insufficient for accurate analysis of urinary calculi. They have failed to identify rare purine stones resulting from genetic disorders such as (2,8)-dihydroxyadenine [14,15].

Moreover, they are unable to quantify each element in mixed stones and accurately distinguish between the different crystal stages of calcium oxalate or calcium phosphate that are associated with very different pathological biochemical and physiological conditions [16]

1-3 previous studies

It has been shown in previous studies in Iraq / Dhi Qar governorate, a high percentage of uric acid and oxalate stones were found, which are more in males than in females, it may be due to the nutritional factor or dairy products and lack of sufficient water intake, which may be the main cause of stone formation [17]

In previous studies analyzed in Iraq/ Anbar Governorate, it was shown that uric acid stones were demonstrated about 50.6% of the total types; Calcium oxalate stone was 38.1%; Calcium phosphate stone was 5.3%; Sistine stone was 1.9%; Blended Stone (Calcium and Polycarbonate Acid) were 4.1% [18]

There is also a study in Tikrit that has been sampled for people aged between 73-23 years. It was found that males are always higher affected than females.

In males, the nature of the diet and environmental factors might be the reason behind increasing their percent. The stone containing calcium and magnesium was more relative than other stones. From the patient's information.

In the United States, the prevalence of gallstone disease is 10% to 15% previous studies showed that about 5% of American women and 12% of men develop kidney stones at some point in their lives; the prevalence is in both sexes. About 80% of the stones consist of calcium oxalate (Ca Ox) calcium phosphate $Ca_3(PO_4)_2$. About 10% of struvite (magnesium ammonium phosphate produced during infection with bacteria possessing the urease enzyme); 9% of uric acid (UA); the remaining 1% consist of cysteine or ammonium acid urate or are diagnosed as drug-related stones [19].

The majority of stones (86%) in a study in Sri Lanka were calcium oxalate [20] inclined to those in India (93%) [21]. In contrast, the proportion of oxalate stones in Jordan is 37.6% [22]; in France 66% [23]; in the United States 26% [24].

The percentage of struvite accounts varies from country to country. The proportion of India is 14% [21] while in Jordan is 13.7%., in the USA, strophe is about 22% of the stones analyzed.

Uric acid stones were found at .93 % in India. In Jordan, this percentage is 20.2%, which may be associated with the high consumption of nuts, which are a rich source of uric acid. In the United States of America, the percentage was about 5%. The results show that the distribution of different types of accounts in Sri Lanka tends to the distribution of India but differs significantly from the distribution of the Western world and Middle Eastern countries. The identification of this pattern is important because it may determine the response to treatment through standard therapeutic maneuvers or plant extracts [25].

1-4: kidney diseases

1-4-1 Renal failure:

Renal failure results from a syndrome (a combination of multiple diseases) that leads to the breakdown of the continuous and non-renewable nephrons, and renal failure begins when there is a gradual decrease in the glomerular filtration rate [26].

Several conditions can cause renal failure, including inflammation of the glomeruli and the urinary tract, as well as other causes such as polycystic kidney disease and high blood pressure [27].

Additionally, the use of medications such as analgesics and their derivatives, some chronic disease such as diabetes, and kidney stone disease, can also lead to renal failure [28].

1-4-2 Kidney stone disease:

Kidney stones and their associated complications are one of the most common diseases. kidney stones and gallstones are the most common types [29].

The term "nephrolithiasis," which means the condition of having kidney stones, comes from the Greek words "nephrons" (kidneys) and "lithos" (stone) [30].

Less common types of stones include pancreatic stones, which may occur chronically, pancreatitis, and salivary stones (usually involving the submandibular gland), which are associated with chronic inflammation of the salivary glands [31].

Kidney stone disease is considered the third most common disease after urinary tract disease and prostate disease [32].

Urinary stones are a common problem due to the geographical location (Iraq is located in the region of the "Stone Belt" that extends from Indonesia to Egypt) [33].

The composition of kidney stones has a direct effect on treatment, secondary prevention, and prediction. Calcium oxalate monohydrate stones are hard and resistant to shock wave lithotripsy, while struvite stones are associated with tissue destruction. The kidneys and infection complications are more common after surgery [34]. The composition of kidney stones varies greatly among populations, even within the same country, and the composition of the stone can be changed according to the geographical region [35]. Therefore, it is useful to know the pattern of stones in the country and region when planning treatment. Typically, stones fill the renal pelvis system, giving the appearance of an antler. Traditionally, stag horn stones are associated with infection stones composed of struvite ($MgNH_4PO_4.6H_2O$), which is the mineral name for magnesium ammonium phosphate. The alkaline urine produced by bacterial infections promotes the formation of struvite [36].

The estimates indicate that at least 10% of the population in the industrialized world is affected by urinary tract stones, with a higher prevalence. Environmental or occupational factors [34], as well as the presence of any gastrointestinal diseases associated with diarrhea or malabsorption, can all contribute to the development of stones.[37]

Also, there are two factors have been assumed: ethnicity resulting from climate, which leads to an increase in urine concentration, and conversion of vitamin D resulting from sunlight, which enhances calcium absorption from food.[38]

About three males are affected for every female. However, there seems to be a changing pattern as the disease has become more common in young females because stones in the urinary tract may be present but without symptoms [39].

The incidence of the disease varies in different regions of the world, with the occurrence rate in Asia ranging from 1-5%, except in Saudi Arabia, where the incidence was higher, reaching 1-20% compared to European countries, where the rate was 5-9%, and in North America (Canada), it reached 12%. The incidence in the United States was 13% [36]. The overall prevalence of kidney disease due to urinary tract stones is about 2-3% in the community per year. In children, the percentage of those affected is 1-2% in developing countries.[40]

1-5: Physical analytic methods for kidney stone

Among the physical methods, X-ray diffraction (XRD) and Fourier Transform Infrared (FTIR) spectroscopic analysis are currently used to analyze kidney stones. Each component is identified and a semi-quantitative assessment of their proportions within the stone is provided.

These methods are capable of identifying non-calcium stones such as cystine, 2,8-dihydroxyadenine, xanthine, uric acid, urates, methyl-1 uric acid, struvite, proteins, fats, or medications, as well as calcium oxalate (CaC_2O_4) and calcium phosphate $Ca_3(PO_4)_2$ stones. Because stones may remain for months or years in the urinary tract, they are common conditions such as primary hyperparathyroidism or type 2 diabetes, or urinary tract infection caused by bacteria that split urea, as shown in figures (1-1a,b,c,d) illustrating stone components for some patients.

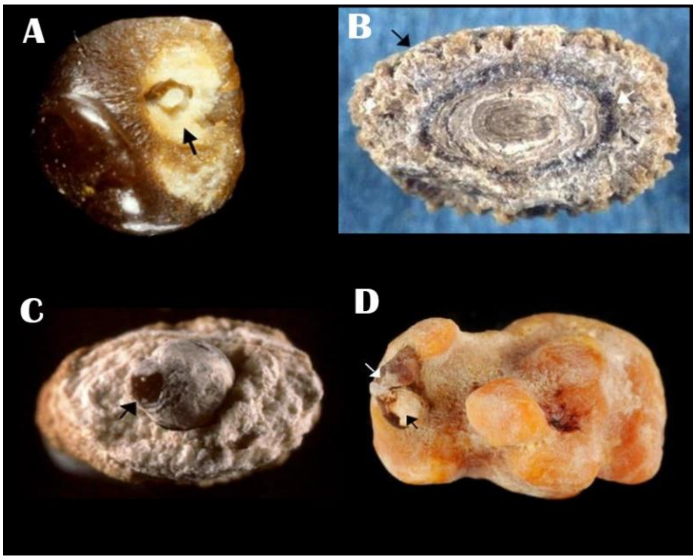

Fig (1-1) shows the different types of the stones [41]

Physical analysis methods provide information about the crystal stages of the same chemical types that may involve in different stones conditions, for example, Calcium oxalate (CaOx), Calcium oxalate monohydrate (COM), and Calcium oxalate Dehydrate (COD) among CaOx stones, and apatite, brushite, or whitlockite among CaPh stones. A similar formation, such as CaOx, may result from a variety of stone processes, including an imbalanced diet, low urine output, and genetic or acquired diseases. This is also true for crystal stages: COM stones may coincide with hyperoxaluria conditions associated with various disease-causing factors, such as primary hyperoxaluria, enteric hyperoxaluria, or unknown cause CaOx kidney stones. On the other hand, COD stones are associated with hypercalciuria in a very high proportion of cases [42]. The corresponding stones show distinctive morphology that can be easily identified on both surfaces and cross-sections (figure 1-2).

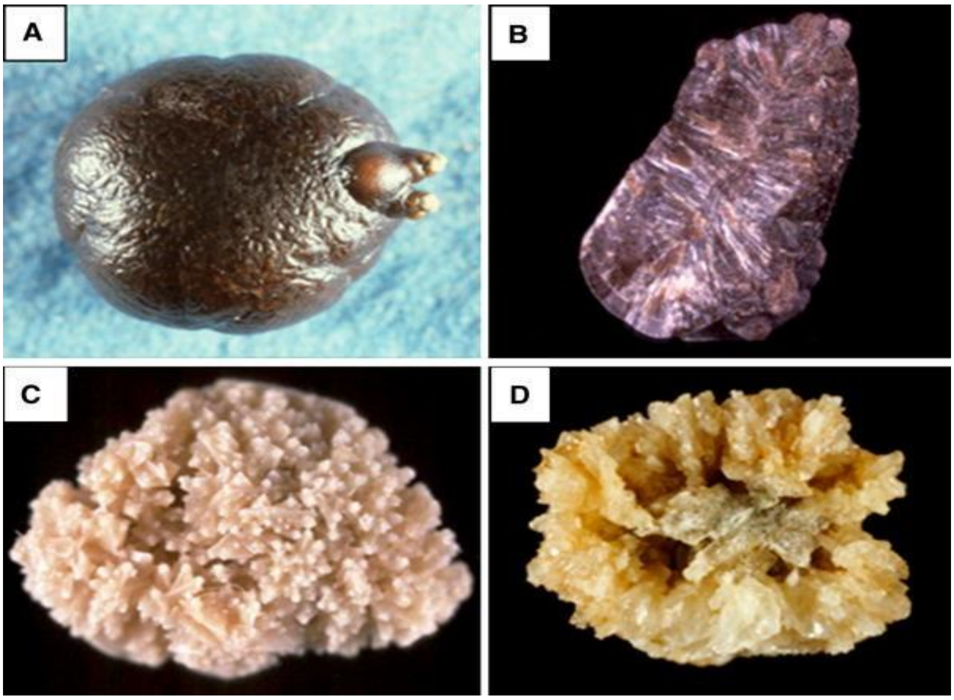

Fig (1-2) shows the different surfaces of the stones [41]

The initial formation process may be linked to other mechanisms (e.g., Randall's plaque) that are responsible for subsequent stone growth. All of these considerations highlight the importance of stone analysis providing information on stone morphology, chemical composition, and crystalline phases, as well as their location within the stone. Only physical methods can determine such diversity in components. For this purpose, numerous techniques have been proposed in routine practice [43], including X-ray powder diffraction (XRD) [44], Fourier transform infrared spectroscopy (FTIR) [45], Raman spectroscopy [46], scanning electron microscopy (SEM) [47] or thermal gravimetric analysis (TGA), as well as solid-state imaging of stone formation. Currently, infrared spectroscopy is widely used for over 300,000 stone analyses annually worldwide. [48]

1-6: The relationship between stone analysis and some other diseases clinically

Stones may be the first manifestation of many metabolic and nutritional disorders. stone analysis is a step to collect all relevant information from the stone to help the physician determine the cause(s) of stone formation and growth. physicians can investigate the biochemical profiles of blood and urine for each previous stone to identify metabolic disorders that can provide accurate information about potential metabolic diseases or risk factors involved in stone formation. However, this metabolic investigation does not guarantee the actual diagnosis of lithogenic disease if the stone composition does not match. Additionally, the stone composition may vary during subsequent analyses in up to 21% of cases [49].

Therefore, in addition to metabolic investigations, the analysis of stones is an essential step in determining the underlying disease. In some cases, the metabolic disorder involved in the formation of stones is not determined by Standard metabolic investigations, while the stone may contain a specific component that allows for a more accurate diagnosis. An example is the deficiency of adenine phosphoribosyltransferase detected by a stone made of 2-8-dihydroxyadenine C5H5N5O2 to obtain this result physical methods of stone analysis are needed [50].

It is necessary to accurately determine the composition of all parts of the stone (the nucleus, inner layers, outer layers, and surface), a comprehensive qualitative analysis of the entire stone (or a portion of the stone) is recommended, with the relative proportions of all components identified by sequencing analysis.[51]

Stones less than 5 mm in diameter have a good chance of passing; those from 5-7 mm have a modest (50%) chance of passing, and those larger than 7 mm always require urologic intervention. Ideally, stone analysis is performed by infrared spectroscopy or X-ray diffraction. The radiographic appearance and density of stones measured by X-ray or computed tomography (CT) scanning are indicative of their precence [52]

1-7: Main Classification of Kidney stones

1-7-1: metabolic stones

Stones that can be formed in the urine include several types, including: calcium stones, uric acid stones, silica stones, drug stones, cysteine stones, and xanthine stones. [53]

2-7-1: Infections stones

Infection stones represent around 30-70% of all types of kidney stones and are associated with bacteria, especially the Proteus genus. Infection stones pose a greater threat than other types of stones, making them more dangerous in terms of kidney damage [54].

1-8: Kidney stones are classified into several types according to their chemical composition.

1-8-1: Calcium Stone

Calcium stones, which include calcium oxalate and calcium phosphate stones, are the most common and widespread type of kidney stone, accounting for about 80% of known types of stones [55]. This type of stone formed due to a disruption in the way the body metabolizes calcium and phosphate, resulting in high levels of calcium in the urine (hypercalciuria) [56]. Other factors that can contribute to the formation of calcium stones include overproduction of the parathyroid hormone (hyperparathyroidism), high levels of oxalate in the urine (hyperoxaluria), or low levels of citrate in the urine (hypocitraturia) [57].

Calcium oxalate salts or phosphates are insoluble salts that form stones, and because of their low solubility, they precipitate and it is difficult to dissolve them. The high saturation state of urine for these salts is a factor responsible for the formation of stones. [58]

1-8-1-1: Calcium Oxalate Stones

Calcium oxalate (CaOx) represents the main chemical types of stones worldwide and can be identified as three different crystalline phases: COM, the most common; COD, country-dependent frequency; and Calcium Oxalate Trihydrate (COT), also called Caoxite, a rare and unstable phase. Comparison of the urinary biochemical chemistry and crystalline phase of recently voided CaOx in urine provided evidence that COM crystals were associated with hyperoxaluria, whereas COD crystals were primarily associated with hypercalciuria. Therefore, COM stones are primarily associated with excessive oxalate concentration (low urine output) and/or excessive oxalate excretion with mild and intermittent hyperoxaluria in 88% of cases, whereas COD stones are associated with hypercalciuria in over 85% of cases [59]

The third form of CaOx (COT) is a rare and unstable form that is observed under uncommon conditions, including hyperoxaluria and specific medication intake. Among CaOx stones, those with high content often contain a mixture of COM and COD and are often associated with varying amounts of apatite (except for Randall's plaque) [46]. In such cases, the same biochemical factors that contribute to each crystal phase, i.e., hypercalciuria, and hyperoxaluria, play a role in stone formation. If there is an increasing amount of carb apatite present, it should be directed towards more specific metabolic imbalances such as bone resorption, Parathyroid hyperactivity, or acidity of the renal tubule [60].

1-8-1-2: Calcium phosphate (Ca Ph)

$Ca_3(PO_4)_2$ is a common chemical component of kidney stones that has been identified in many types of stones. The clinical importance depends on the crystal phase, location, and overall content within the stone. The increasing portion of apatite Randall's plaque has been previously confirmed as a nidus for CaOx stones. In such cases, the CaPh content is low (<5%) [61]. Other causes of CaPh should be considered. Since carb apatite depends heavily on pH, it is expected that stones rich in carb apatite are developed in alkaline or low-acidic urine. This indicates urinary tract infection (UTI) or metabolic disorders responsible for chronic urinary acidification, associated with hypercalciuria [62]. Carbapatite is usually associated with brushite and/or octacalcium phosphate pentahydrate $Ca_8H_2(PO_4)_6.5H_2O$ (OCPP) as a sign of hypercalciuria.[16]

Urinary tract infection is one of the most common mechanisms that produces phosphate stones [63]. In these cases, several characteristics of the stone should be taken into account:

1- In addition to carbonate, other types of CaPh exist in the stone, particularly non-crystalline carbonated calcium phosphate and/or whitlockite.

2- A specific hallmark of stones caused by urinary tract infections is the presence of struvite.

3- If struvite is not present, another useful sign may be the carbonate apatite content as determined by infrared spectroscopic analysis [64]. When it exceeds 15%, the probability that urinary tract infection is a driving force for stone formation is very high. Rarely is the apatite pure, and other crystalline stages present as secondary components may help to identify the diagnosis. COD-associated carb apatite strongly suggests hypercalciuria and should raise suspicion of primary hyperparathyroidism [65] In contrast, COM-associated carb apatite is more strongly associated with medullary sponge kidney and other causes of urinary stasis.

One of the metabolic causes of calcium-rich kidney stones is considered to be hypercalciuria, and its mechanism is often absorptive and/or resorptive [61]. Primary hyperparathyroidism is one of the main causes of absorptive hypercalciuria. Recently, kidney stones related to this medical condition have been investigated, revealing unique features in stone composition Fig (1-3) [66].

Fig (1-3) Examples of brushite stones type IVd (left surface; right section [41]

A rare cause of calcium phosphate stones is impaired protein excretion by kidney cells as observed in a defect of the distal renal tubule or acquired autoimmune diseases. Urinary stones also consist of carb apatite with a very high content of calcium phosphate; they are often higher than 80% of the mass of stones Cases (figure 1-4) [67]. These results emphasize the importance of structural analysis to assist in clinical diagnosis.

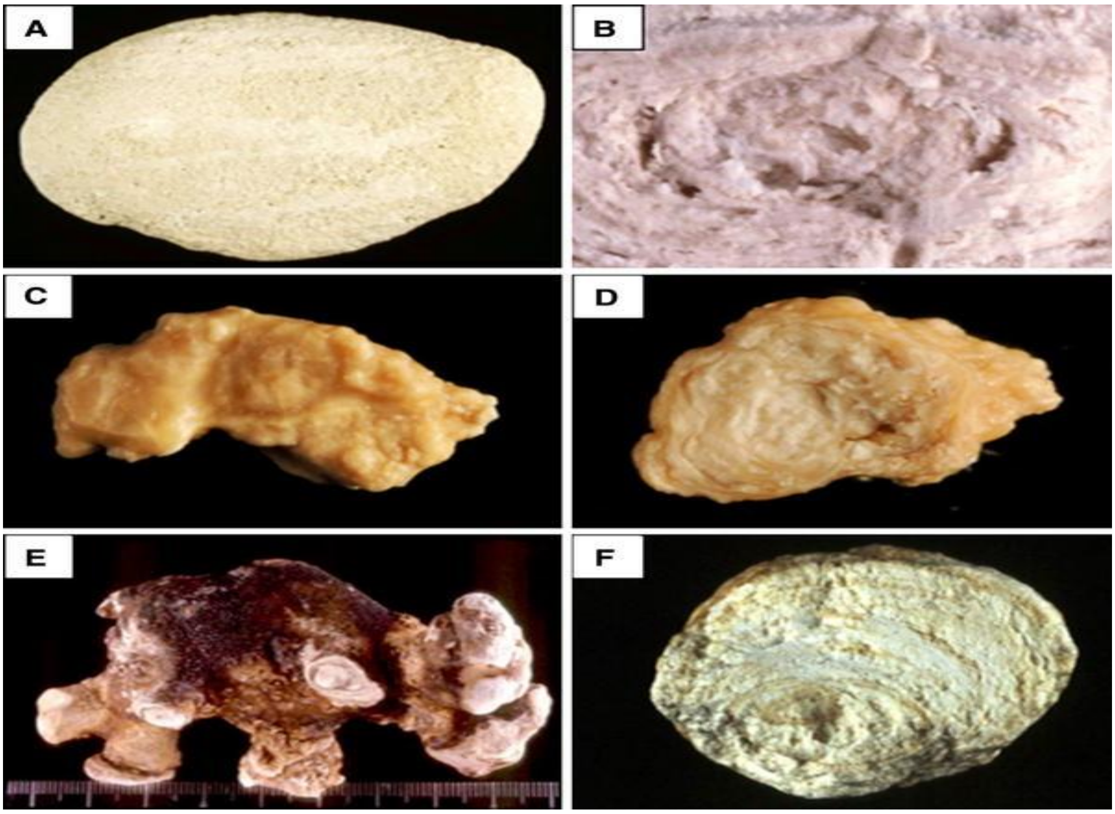

Fig (1-4) shows the different shapes of kidney stones and illustrates their structural composition [41]

COM Stones represents the most common and abundant component of stones in most countries around the world [68-71]. The morphological aspect of COM stones directs toward various diseases or lithogenic conditions.

- Mild intermittent hyperoxaluria associated with high oxalate intake

- Reduced urine output with increased concentration of oxalate ions in the urine

- Heavy hyperoxaluria is either associated with genetic diseases (primary hyperoxaluria type 1) or with enteric hyperoxaluria [72]

COM stones exhibit five different shapes in the first category of constitutional formation classification.[73]

The subtype IA (1-5), which is often dark brown, indicates slow and intermittent growth associated with peaks of excessive urinary calcium excretion (decreased urine output or consumption of calcium-rich foods). It is the most common subtype of calcium stones in most countries.

When a thin gray layer is observed on the surface of a stone, it corresponds to recent secondary COM crystalline deposits from a recent concentration of urinary oxalates (Fig 1-5).

Fig (1-5) subtype IA of COM stones [41]

The stone type in figures (1-5) is a subtype IA of COM stones. Note the thin gray layer of newly deposited crystals that covers the brown surface of the stone. This gray coating is produced by a recent bout of excessive oxalate in the urine, often related to consuming oxalate-rich food. The subtype IB in figure (1-6) could be a sign of an older stone, perhaps first developed as a satellite stone due to transient hypercalciuria. Both subtype IA and IB stones often have a dark brown color.

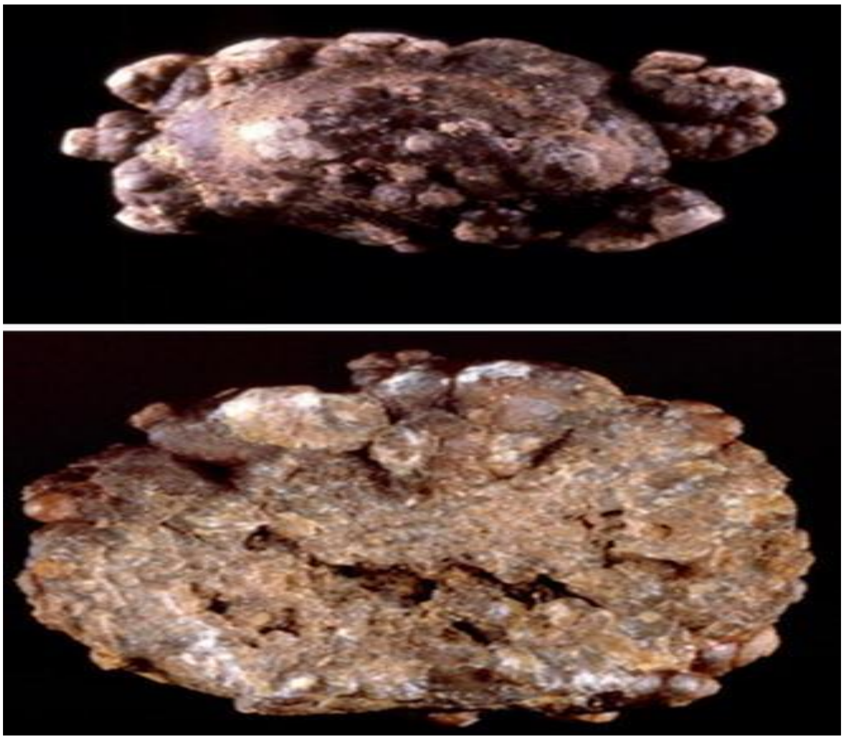

Fig (1-6) is a subtype IB of COM stones [41]

In the IC subtype, the color is very light, brown-yellow pale, or even white in children's shape (1-7).

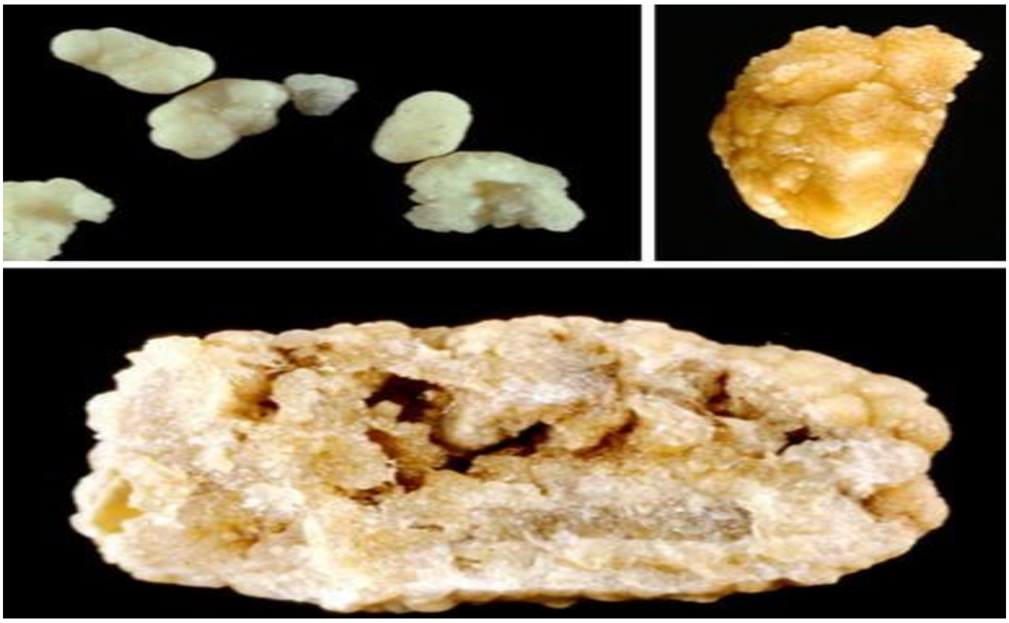

Fig (1-7) IC type in children [41]

It is associated with heavy hyper oxalate, mainly type I hyper oxalate (associated with the deficiency of aminotransferase glyoxylate in liver cells), the most severe measles disease that is often responsible for the final stage of kidney failure, especially when a diagnosis is delayed [74]. Oxalate in the body utilizes two sources: internal and external

A- Endogenous oxalate

40-50% of oxalates in the human body are produced during the metabolic process (glyoxylate) in the liver [75].

B- Exogenous oxalate

Oxalates are produced by consumable plants such as Rhubarb, tea, coffee, fruits, vegetables, spinach, cactus, sorrel, chocolate, desert lily, black pepper, and broomrape [76].

Most kidney stones are formed from calcium oxalate and are influenced by various factors, including environmental and genetic factors and a diet rich in oxalates. A diet rich in animal protein, excessive sweating, and intestinal disorders can lead to increased oxalate levels in the urine. However, in healthy individuals, internal sources of oxalates are reduced [77].

1-8-2: Uric Acid Stone

Uric acid stones constitute approximately 5-10% of kidney stone types are among the most common types of stones in industrialized countries and communities with high protein intake [78]

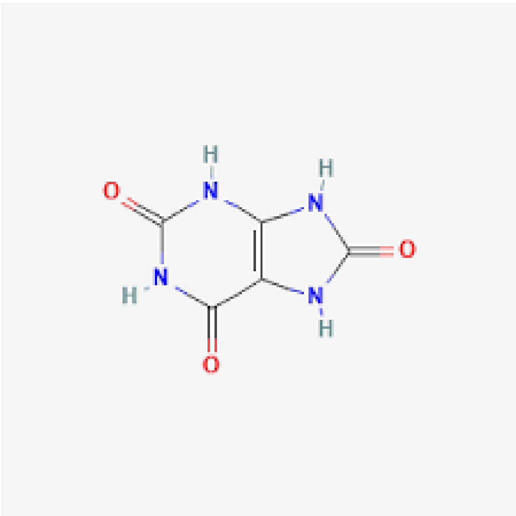

Fig (1-8) chemical structure of uric acid [79]

The primary composition of the stone is uric acid $C_5H_4N_4O_3$, which is a final product of purine metabolism in nucleic acids. [80].

The limited solubility of uric acid in the water, especially in an acidic environment, low urine volume, high acidity with a pH of 5.5 or less, and hyperuricosuria can lead to the formation of uric acid crystals, which cause this type of kidney stones in the urinary tract [81].

1-8-3 Struvite Stone

Bladder stones are composed of ammonium magnesium phosphate ($MgNH_4PO_4.H_2O$), which is formed from struvite, as well as carbonate apatite (Ca_{10} PO_4, CO_3). Bladder stones can be formed from either one of these minerals or a combination of both.

Stones are only formed in the kidneys and urinary tract when the urinary tract is infected with bacteria that produce enzymes that break down urea, such as Proteus bacteria that produce the enzyme Urease

Struvite stones tend to branch out and expand, and their growth is usually rapid. They typically fill the collecting system of the kidney, taking on the shape of a stag's horn (Staghorn) [82].

This type of stone is considered the most common among other types of stones, as they tend to regrow after surgical removal due to the presence of remaining infected calcified tissue in the area [83].

1-8-4 Cystine Stone

Cystine stones are the least common type of kidney stones, accounting for about 1% of all kidney stones. This type of stone is formed due to the excessive excretion of cystine in the urine, which is a result of a rare genetic disorder affecting the transport and absorption of amino acids such as cystine, lysine, ornithine, and arginine [84].

During renal tubules and intestinal epithelial cells, the solubility of this acid in diuresis is approximately (300) mg / L and its solubility increases with increasing pH with a dilution of urine [85] Fig (1-9) Show the chemical structure of Cystine

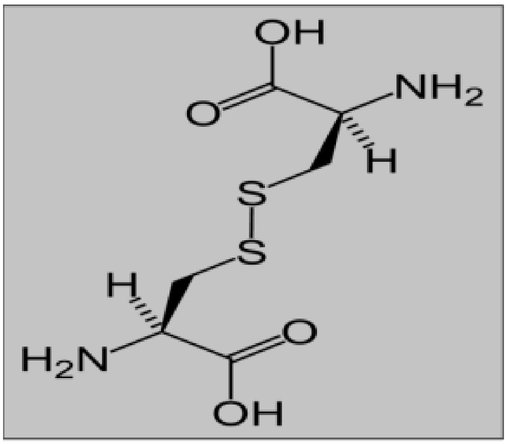

Fig (1-9) the chemical structure of Cystine [86]

1-8-5 Rare Stone

These are rare types of kidney stones that are formed under specific conditions, and there are several types of them [87]:

Silica stones, 2,8-Dihydroxyadenine stones, Xanthine stones and Drug-induced stones.

1-9- Formation of kidney stone

Kidney stones are defined as solid masses formed by small mineral crystals that are similar to rocks, and they can be formed or exist in the kidney or urinary tract [88]. They are also formed by the deposition of mineral substances from a highly saturated solution, and most kidney stones, especially small ones, pass out of the body through urine. Their movement and descent are usually accompanied by severe pain and may obstruct the urinary tract if they are large, leading to a decrease in urine output if they become trapped.

In the ureter, it has caused severe renal colic with lower back pain as well, which requires surgical intervention to remove it [19]. Other symptoms include nausea, vomiting, purulent urine, pyuria, blood in the urine, hematuria, obstruction of urine flow, or lack of oliguria due to obstruction of the bladder.[89]

And the ureter, as well as the urethra with stones, with a constant desire to urinate and burning in the urine when the stones come down, and thus the urine becomes cloudy and smelly in addition to fever, weakness chills loss of appetite, and weight loss [21].

Kidney stones do not have a single well-defined causes but they are the result of a combination of factors. Stones form when urine does not contain the proper balance of fluids and a variety of minerals and acids [90].

Kidney stones or calcium oxalate crystals in the kidneys can be caused by underlying metabolic conditions, such as renal tubular acidosis [91], Dent's disease [92], hyperparathyroidism [93], primary hyperoxaluria [94], and medullary sponge kidney [95]. Stones are also more common in patients with Crohn's disease [96]. Patients with recurrent kidney stones should be evaluated, typically by collecting urine for 24 hours and analyzing it chemically to detect deficiencies and excesses that promote stone formation.[97]

There has been confirmed that fluoridated water may increase the risk of kidney stone formation [98]. Vitamin C can also cause kidney stones, which underscores broader concerns about the efficacy of vitamin supplements. A recent study suggested a possible causal relationship, but the evidence was not conclusive [99]. The American Urological Association has predicted that global warming will lead to a greater prevalence of kidney stones in the future, particularly by expanding the "kidney stone" [100]. It also appears that astronauts are at increased risk of kidney stones during or after long space flights [101].

Kidney stones can also be formed as a result of various conditions, in which biological fluids are in a state of saturation or supersaturation, known as the metastable region. This leads to the deposition of salts in this region and the formation of stones.

Deposition occurs due to the availability of two conditions: first, the solution remains still for a sufficient amount of time to allow the formation of stones, and second, a material acts as a nucleus available around which the stones form. This stage is influenced by many factors such as temperature, availability of ions, and the flow rate and turbulence of the fluid. The important factor here is the concentration of solutes in the fluid. After this stage, which is called the metastable region, the crystals present begin to deposit, whether a nucleus is present or not, this stage is called nucleation. Stones are not formed solely from crystallized material but have their unique composition. There is organic material present within the crystallized material, and the material then deposits in the form of rings, similar to the rings of tree growth. Thus, two basic components can be distinguished for each stone: the first is the scaffold, which is the organic material, and the second is the filler, represented by the crystallized deposit [102].

Kidney stones can also occur due to various factors such as disrupted metabolism, infections, hormonal imbalances, environmental and dietary habits, and low fluid intake leading to concentrated urine and consequently decreased urine volume. This can also occur due to urinary bladder obstruction or high excretion of chemicals such as calcium, magnesium, oxalates, carbonates, phosphates, urates, xanthines, cystine, and so on. It is one of the most painful urinary tract diseases.[103]

Kidney stones may contain various groups of chemicals that are a part of a person's natural diet and are also present in bones and muscles [104]. The likelihood of kidney stones recurring is very high. In a survey by Anderson DA, the recurrence rate of kidney stones was 60% in 7 years and 80% in 18.5 years [105].

1-10: Kidney stone formation theories

There are multiple theories explaining the mechanism of kidney stone formation, some of which are more accepted, including:

1-10-1 Precipitation Theory

This theory is based on the increase in the concentration of waste products in the urine to a level super saturation, causing them to lose their solubility and ultimately leading to the deposition of salt crystals that grow and form kidney stones [106].

1-10-2 Leak of Inhibitors Theory

The balance between the presence of inhibitors in the urine and the crystallization process is a force that works against the formation of kidney stones. Compounds such as citrate, magnesium, pyrophosphate, and amino acids act as inhibitors by forming soluble complexes with calcium or oxalate ions, reducing their ion activity and thus preventing the transition from the post-nucleation stage to the nucleus formation stage [107].

1-10-3: Matrix Theory

The Matrix is an organic proteinaceous substance that is involved in the formation of many kidney stones, and its content usually varies between (10-2) % of the stone's weight [`108]. Some urinary proteins such as the Uromucoid can promote the deposition of calcium phosphate or calcium oxalate [109].

1-11: Factors affecting the stone formation

1-11-1: External factors affecting stone formation:

A- Gender

Research has shown that men are more affected than women to develop kidney stones [110]. by (3:1) but the formation of infection stones is more common in women than in men [111]. The high number of infections compared to women is probably due to the high osmolality of urine in men compared to women [112]. The lack of gravel formation in women may also be due to the presence of estrogen in women which reduces the saturation of gravel-forming salts [113].

B- Age

Most urinary tract stones occur in individuals aged between 20 and 61 years [114]. For those who experience recurrent stone formation, their first episode of kidney stones typically occurs during adolescence or early adulthood, and it is believed to be relatively uncommon for stones to develop for the first time after the age of 50 [115].

C- Race

Research has shown that Caucasians are more susceptible to kidney stones than people with darker skin tones, such as African Americans [116]. The reasons for this phenomenon are not fully understood, but it is believed to be influenced by genetic factors, geographical location, and dietary habits [117].

D- Genetic

Most epidemiological studies agree that over 40% of kidney stone patients have a positive family history, making the affected individual three times more likely to develop urinary tract stones [118].

E- Geographical factors

There may be a strong correlation between geographical factors and the prevalence of kidney stones. For example, in the Kingdom of Saudi Arabia, there is a clear increase in the incidence of kidney stones with rising temperatures [119]. It is believed that dehydration leads to an increase in urine concentration and therefore an increase in the formation of kidney stones. However, in other parts of the world such as South Africa, where the climate is hot, this factor does not affect the formation of kidney stones as they are relatively low [120].

F- Kidney stone-stimulating diseases

1- Idiopathic Hyperparathyroidism:

Idiopathic Hyperparathyroidism might be results in excessive presence of calcium in urine (hypercalciuria) in 5-10% of kidney stone cases [121].

2 - Dehydration:

Dehydration refers to the process of removing water from the body, which occurs as a result of excessive food intake (dietary excess) or excessive intake of certain medications (drug excess) [122]. In both cases, the body consumes more water than normal, leading to a decrease in its natural levels, which in turn leads to an increase in salt deposits in the urine [123].

3 - Diet:

Eating food high in oxalates can affect the levels of oxalates in the urine. Consuming large amounts of oxalates can lead to excessive absorption by the intestines due to the low availability of calcium, leading to the formation of kidney stones [124]. Some types of food contain compounds that play an important role in the formation of kidney stones, including:

4 - Calcium:

One of the causes of calcium stone formation is an increase in calcium concentration in the urine, which accounts for the vast majority of kidney stones. Therefore, patients are advised to reduce their intake of food rich in calcium to prevent this increase in concentration. Some studies have confirmed that reducing the consumption of food that contains calcium, such as dairy products, fish, figs, chickpeas, salmon, and okra, leads to increased absorption of oxalates by the intestines, which ultimately leads to excess oxalates in the urine [125].

5 - Oxalates:

Several types of food are a source of oxalates, such as spinach, hazelnuts, wheat bran, peanuts, tea, and chocolate. The absorption of oxalates occurs through the intestines, depending on the concentration of calcium in them, meaning that an increase in calcium concentration reduces the intestines' ability to absorb oxalates [126].

6 - Magnesium:

Previous studies have shown that magnesium reduces the growth and formation of calcium oxalate crystals. Also, the binding of magnesium with oxalates in urine or intestines creates a more soluble compound than calcium oxalate, which reduces the saturation of urine with calcium oxalate [127]

7 - Sodium:

It is one of the factors responsible for the formation of kidney stones, especially calcium stones. An increase in the amount of sodium consumed in the diet increases the likelihood of stone formation, and the lower the proportion of sodium in the diet, the less likely the formation of stones. Sodium secretion increases through the renal tubules of calcium secretion in urine. Therefore, restricting dietary salt, which reduces sodium secretion, is associated with a decrease in calcium secretion. If sodium secretion remains high, patients should be encouraged to reduce sodium intake, and thus, the addition of sodium (such as sodium bicarbonate) increases the formation of sodium urate, which acts as a nidus for calcium oxalate deposits at high acidity. Increased salt intake enhances a variety of effects, as it promotes calcium secretion in urine, increases the rate of kidney stone formation, increases the acidity of urine, and reduces the secretion of urinary citrates [128]. Patients should aim to limit their sodium intake to 3000mg per day to lower the concentration of calcium in the urine [129].

8 - citrates

The citrates are considered as factors that inhibit the formation of stones by approximately 50% of urinary calcium, as they bind in complex auto-associations, thereby reducing the ionic concentration of calcium and the relative saturation of calcium oxalate and phosphate in the urine. [63, 130].

9-Phosphate:

Phosphate contributes to the formation of stones and this depends on an increase in its concentration in the urine [63].

10-Protein:

A diet rich in protein increases the likelihood of stone formation due to an excessive concentration of uric acid, calcium, and acidity in the urine, as in people who consume a lot of meat [131].

1-11-2: Internal factors that affect stone formation include:

1 - pH level:

The pH level plays an important role in the formation process as its increase or decrease affects the relative saturation of urine components and hence their precipitation, indicating the transition from the post-steady state to the nucleation phase [132] as shown in the table below (1-1).

Table (1-1) shows PH Acidity and alkalinity for types of kidney stones

Acidic PH Crystals	Alkaline PH CRYSTALS
Calcium oxalate	Amorphous phosphate
Uric acid	Triple phosphate
Amorphous urate	Calcium carbonate
Leucine	Ammonium bitrate
Tyrosine	
Cystine	

2- The concentration of soluble substances in urine:

Calcium, phosphate, oxalate, protein, and uric acid, are considered promoters of stone formation [123]. On the other hand, substances such as citrate, magnesium, pyrophosphate, and amino acids act as inhibitors of stone formation [133].

3 - Urinary tract obstruction:

Enlargement or congenital deformities of the prostate gland can lead to urinary stasis, which increases the likelihood of salt deposition in the urinary tract, especially in the ureter and bladder, leading to the formation of stones [134]. Researchers have pointed out a clear relationship between urinary tract obstruction and the formation of stones, which is caused by bacteria that produce the enzyme that breaks down urea, including Proteus Spp [135]. Recently, a group of Nanobacteria has been observed in more than 90% of cases of kidney stones [136].

1-12- Aim of the study

1-Estimation the chemical components of kidney stones in patients among Karbala Governorate.

2- Comparing the chemical components of kidney stones in Karbala governorate with the chemical components of kidney stones for patients in other governorates.

3- select the area's most capable to kidney stones.

4- select the age and sex most susceptible to the disease.

Chapter Two

Materials and Methods

2-1: The chemicals

The Chemicals used in this study was listed in Table (1-2)

Table (2-1) Chemicals used and their general formulas and manufacturers:

Material	Chemical formula	molecular mass	Percentage of purity	the manufacturing company
nitric acid	HNO_3	63.01	65%	Fluka (England)
hydrochloric acid	HCl	36.46	50% v/v	Fluka (England)
Sulfuric acid	H_2SO_4	98.079	98% v/v	Fluka (England)
hydrogen peroxide	H_2O_2	34.01	50% v/v	B.D.H(England)
potassium permanganate	$KMnO_4$	158.034	99%	B.D.H(England)
manganese sulfate	$MnSO_4$	151.001	99%	H.W (England)

2-2: devices used

Table (2-2) devices used

Sequence	the device name	Location of the device	the manufacturing company
1	IR Spectrophotometer	Karbala University \ College of Education for Pure Sciences \ Department of Chemistry	Shimadzu-Jaban
2	Flame atomic absorption device	Karbala University\College of Medicine	SHIMADZO-JAPAN

3	Sensitive balance	Karbala University \ College of Education for Pure Sciences \ Department of Chemistry	Kern-Germany
4	Hot plate	Karbala University \ College of Education for Pure Sciences \ Department of Chemistry	Kern-Germany

2-3: Sample collection

Kidney stone samples (35sample) were collected from patients with kidney stones in hospitals in Karbala Governorate after surgery for eradication in the Department of Urology. Data were taken on the condition of patients ranging in age from 13 - 70 years. The samples were transported with clean equipment and washed with distilled water and blood and other materials were removed from them. and transferred to work in the laboratory.

2-4: Stone analysis set

2-4-1 Working principle

Determination of the main mineral components and the organic component (cysteine) of urinary tract stones using simple chemical tests. [137]

2-4-2 The reagents used [137]

Table (2-3) Shows the reagents used

Vial R1	Hydrochloric Acid (HCl 1.65 mol\L)
Vial R2	Sodium Hydroxide (NaOH 6.25 mol\L)
Vial R3	1st Reagent for Cystine determination (NaOH, Sodium Cyanide)
Vial R4	2nd reagent for Cystine determination (Na_2[Fe (CN)5]NO

	Sodium nitroprusside)
Vial R5	Reagent for Phosphates determination (Sulfuric Acid H_2SO_4, Ammonium Molybdate $(NH_4)2MoO_4$, Ferric Sulphate $Fe_2(SO_4)_3$)
Vial R6	Reagent for Magnesium determination (NaOH, paranitrophenylazoréesorcinol
Vial R7	Reagent for Calcium determination (KOH, calcium)
Vial R8	Reagent for Ammonia determination (Potassium Iodide KI, Mercuric Iodide Hg I_2
Vial R9	Reagent for Uric Acid determination (Acetic Acid CH_3COOH, neocuproïne $C14H_{12}N_2$, Copper Sulphate Cu SO_4)
Vial R10	Reagent for Oxalate determination (Manganese dioxide Mn O_2)

2 -4-3 working method [137]

1-Weigh 50 mg of stone powder after grinding it with an earthenware mortar, then transfer it to a test tube and add 10 drops of reagent R1. Fizzing appears, which is evidence of the presence of carbonates, as proven in the table below in this case. Shake vigorously for 1 minute. The remaining mixture is called M1.

2- Prepare a mixture of M2: mixing 50 µL of M1 and 5 mL of distilled water Mix well and only used for the determination of calcium (step number 5).

3- Distribute one drop (about 50 µL) of the mixture M1 and M2 in all tubes and perform the following tests (Steps 2 to 8).

Table (2-4): The method of action for the analysis of stones using the stone analysis set [137]

Step1		Step 2	Step 3	Step 4	Step 5	Step 6	Step 7	Step 8
Carbonate Prepare 50 mg of gravel powder + R1 10 drops (500 µL) = M1		cystine	phosphate	magnesium	Calcium	ammonium	uric acid	oxalates
		M1 1 drop	M1 1 drop	M1 1 drop	M2 1 drop	M1 1 drop	M1 1 drop	The remaining mixture
		+ R3 1 drop	+ R5 2 drop µL 100	+ R6 1 drop	+ R2 1 drop	+ R2 1 drop	+ R2 1 drop	+R10 60mg almost
		Mix and keep it for 5 minutes	Mix and keep it for 5 minutes	+R2 5 drops (250 µL)	+R7 2 drops (100 µL)	+R8 1 drop	+R9 1 drop	
		+ R4 1 drop	Mix ↓	Mix ↓	Mix ↓	Mix ↓	Mix ↓	Wait for a few seconds
Positive result	Visual and audible effervescence	Color: Red	Color: Blue	Blue precipitate	Color: Yellow	Orange to brown precipitate	Yellow to orange color	Visual and audible effervescence
Negative result	No effervescence	Color: Yellow	No discoloration	No delicate purple color	Orange color	Clear light yellow	No discoloration	No effervescence

In this step, the type of pebbles is detected, and the same stone may consist of two components, The rest of the pebbles were taken to perform the rest of the quantitative analysis.

2-5 Quantitative analysis

2-5-1: Preparation of solutions

1 – Prepare a 50% (v/v) solution of hydrochloric acid (HCI):

$$\frac{V\ Hcl \times 100}{V\ Hcl\ + Vwater}$$

$$\frac{50}{50 + 50} \times 100 = 50\%$$

2 - Prepare a 2N Solution of sulfuric acid (H_2SO_4):

N =$(Density * percentage * 10)/(eq.wt)$

N= $(1.84 * 98 * 10)/49$

N=36.8

$N_1\ V_1 = N_2\ V_2$

$36.8 * V_1 = 2 * 1000$

$V_1 = 54.94$ ml

3 - Prepare a 5% (w/v) Solution of manganese sulfate (MnSO4):

This solution is prepared by dissolving 0.5 g of manganese sulfate in 10 mL of distilled water.

4 – Prepare a 0.01 M of potassium permanganate:

prepared from a solution of 0.01 M of potassium permanganate by dissolving 0.315 g of permanganate in a liter of distilled water.

2-5-2: Estimation of calcium oxalate by titration using the method Hodgkinson A [138]

1- Dissolve 20 mg of gravel powder in 2 mL of 50% (v/v) of HCl acid, then dilute to 10 mL with distilled water in a volumetric flask (solution A). This solution is then used and the gravel solution is kept at a temperature between (2-8 ċ) when not used.

2- Mix 1 mL of the dissolved stone (solution A) with 2 mL of 2N H2SO4. Add one drop of an aqueous solution of 5% (w/v) MnSO4(This solution is prepared by dissolving 0.5 g of manganese sulfate in 10 mL of distilled water) The resulting solution is heated between (70-80 °C,)

3- The solution prepared in step 2 was titrated with a standard solution of 0.01 M of potassium permanganate until the pink color of the permanganate proves, indicating the end of the reaction (it is prepared from a solution of 0.01 M of potassium permanganate by dissolving 0.315 g of permanganate in a liter of distilled water).[139]

The percentage composition of a stone in terms of calcium oxalate can be calculated from the

following equations:

1mL of 0.01M $KMnO_4$ = 0.45 mg of oxalic acid

Weight of Calcium oxalate monohydrate = oxalic acid (grams of anhydrous acid per 100 gm. of calculus) ×1.62 ………. (1)

number of milligrams of oxalic acid per1 milligram of gravel = Titration volume x 0.45 x 1/2 ………. (2)

Weight of Calcium oxalate monohydrate = product of equ (2) x 100 x 1.62

Weight of total calcium = $weight\ of\ calcium\ oxalate\ monohydrate\ x\ 40 \div 146.12$

40 = Calcium atomic number

1.62 = Molecular weight of calcium oxalate monohydrate\ The molecular weight of oxalic acid anhydrous

2-5-3: Measure of magnesium

Using a flame atomic absorption device to measure magnesium concentration to estimate struvite stones.

Before using this device, the sample must be converted from solid to liquid to be measured with this device. Therefore, the digestion process was carried out according to the following method: [140]

1- take 0.1 g of the form (crushed gravel), put it in a crucible, and add 10 mL of concentrated HNO_3 acid.

2- The crucible is placed on the hotplate for 30 minutes at a temperature of 70 °C. Then we cool it.

3 – 4 mL of a solution (20%) of H_2O_2 is added until the solution becomes clear and close to dry.

4 - After cooling, the filtrate is filtered and transferred to a 25 mL volumetric flask and diluted with deionized distilled water, after which it is stored until it is used for elemental Mg measurement in a flame atomic absorption device.

This device was used for samples that proved the presence of the element magnesium at the University of Karbala, College of Medicine, and the concentration of the element was measured by the person in charge of the device.

Fig (2-1) Flame atomic absorber device used

2-5-4: Infrared spectrophotometer

Pulled infrared spectrometry of kidney stones in the range (400-4000) cm-1 with a typical device (Bruker FTIR - Spectrometer) and using potassium bromide disk at Karbala University/College of Education for Pure Sciences/Department of Chemistry.

Approximately 0.01gm of gravel powder was taken and the samples were examined by infrared spectroscopy to find out the effective totals for each sample and compared with the rest of the extracted results

2-6: Statistical Analysis

Information from the form for all participants were entered a data sheet and were assigned a serial identifier number. Multiple entry was used to avoid errors. The data analysis of this work was generated using The Statistical Package for the Social Sciences software, version 28.0 (IBM, SPSS, Chicago, Illinois, USA).

Descriptive statistics was performed on the participants' data of each group. Values were illustrated by n (%) for categorical. The distribution of the data was checked using Shapiro-Wilk test and boxplot as numerical means of assessing normality.

Significant differences in categorical variables among the parameters were confirmed through analytical statistical tests. Results of all hypothesis tests with p-values <0.05 (two-side) were considered to be statistically significant

Chapter Three

Results and Discussion

3- Results and Discussion

Knowing the chemical composition of kidney stones is essential to know the causes of the disease. The treatment of kidney stone diseases is based on chemical analysis of the stones, and it can also prevent recurrence or recurrence of stone formation.

The study included the analysis and of kidney stones, The color and weight of these stones, the sex and age of affected patients were statistically estimated, as well as the investigated of their chemical components using different analysis methods such as the use of easy chemical tests (KTAT), titration, and infrared spectroscopy technique, the results were as follows the results are as follows:

3-1: color

Table (1-3) shows the colors of the pebbles, which could give an idea of the components of these pebbles, especially the pure types. The difference in these colors was due to the components of the pebbles and the crystalline phases that caused their formation [45]

Table (3-1) Stones colors and their relationship to components

Colors	Compounds
Blackish brown	Calcium oxalate monohydrate
Dirty white	Struvite
Whitish brown	Ammonium ion calcium oxalate monohydrate
Dark brown	The mixture of calcium oxalate monohydrate and calcium phosphate
Yellow	Uric acid

3- 2: Weight

The weights of the grafts obtained from the surgeries varied as the weights ranged from 0.06 - 15 g. The reason for this large size may be due to the formation of stones for a longer period when they are formed inside the kidneys and neglected by the patient or due to metabolic disorders that contribute to the formation of this size and shape.

3-3: Gender

The study showed that the number of affected males were 20 samples and the number of affected females were 15 samples as shown in the table (3-2) this result is agreed in most studies conducted in many countries, that might be due to the large muscle mass of men compared to women; the daily diet also produces an increase in metabolic waste, as the male urinary tract is more complicated than the female urinary tract. On the other hand, the hormone estrogen has a major role in women, it reduces the saturation of oxalates in the urine, also sweating and eating meat is more often in men than in women [100].

Table (3-2) the relationship of gender with the number of people with stones

Gender	Number of participants	%
Female	15	43%
Male	20	57%

3- 4: Age

After classifying patients into three age groups, our study showed that the prevalence of kidney stones was high in the age group (>50) years (52%). While the ages of (32-49) years the percentage was (34%) and the lowest among the ages (13-31) (14%) as shown in the table (3-3). this matches with a study conducted by (Rahman 2014) who reported that older people have higher rates of morbidity from kidney stones and higher risk of infectious complications and comorbid conditions such as diabetes, which can increase uric acid stone formation. Also, most of them are taking medications and vitamin supplements which change their metabolic profile and increase their susceptibility for stone formation. Also, Obesity, a body mass index (BMI >30 kg/m2), affects more than 300 million people round the world. It is believed that overweight, obesity and larger waist circumference are risk factors for calcium oxalate and uric acid renal stone formation. In addition, stone formation may be a marker for increased risk of chronic kidney disease and cardiovascular disease, which are particularly important in older ages. In patients over 70 years of age, 26% has evidence of estimated glomerular filtration rate (GFR) <60 mL/min [141].

Table (3-3) Shows the Distribution of stones by age

Age Groups	Number of cases	(%)
Group 1 (13- 31)	5	14.3
Group 2 (32-49)	12	34.3
Group 3 (>50)	18	51.4

3-5: Diagnosis of stone components using stone analysis set.

Table (3-4) shows exactly the types of the chemical composition of stones in the patient's group, and the results indicated that the predominant percentage among the patient's stones were calcium stones were about 28 samples, which is the most common type of stone, as in the USA study [142], where calcium stones account for about 80% of urinary stones. Changes in eating habits, the environment, or both can lead to an increase in calcium stones. The formation of calcium stones is associated with a malfunction in the calcium-phosphate metabolism in the body, hence the occurrence of hypercalcemia in the urine (source), hyperthyroidism, hyperoxaluria, or a low level of citrate in the urine

Oxalates are in second type, about 20 samples were the cause of the formation of this type of stone-hyperoxaluria and hypercalciuria. Uric acid stones then come with about 12 samples. the formation of uric acid stones is associated with excessive uric acid in the urine and continuously low urine pH as well as diabetes and obesity performance which significantly increases the risk of stone formation [143].

Table (3-4) Total Chemical qualitative analysis of the Stone composition

Type of Stones	Number of samples	Total %	Total Mean level
Uric Acid Stone	12	34	17
Cystine	1	3	20
Phosphate	9	26	5
Magnesium	4	11	1
Oxalate	20	57	11.6
Calcium	28	80	10.86

In table (3-5), the chemical qualitative analysis of the stone composition was performed based on gender. Results indicated that the mean levels of Uric Acid, Oxalate, and Calcium in kidney stones were higher in female patients than in males. In females, the mean level was 19.16mg, 12.85 mg, and 11.15 mg whilst in males was 15.55 mg, 11.07 mg, and 10.62 mg respectively. On the other hand, the mean levels of Phosphate and Magnesium were constant in both, 5mg and 1mg in each male and female patient. Interestingly, Cystine level in the kidney stone was shown the lowest percent and appeared in male patients only.

Table (3-5) The Chemical qualitative and quantitative analysis of the stone composition with the mean differences level of each type based on gender groups

Type of Stones	% In Female	% In Male	Mean Level / Male	Mean Level / Female
Uric Acid Stone	46.7	30	15.55	19.16
Cystine	0.0	5	20	\
Phosphate	26.7	25	5	5
Magnesium	6.7	15	1	1
Oxalate	53.3	60	11.07	12.85
Calcium	86.7	75	10.62	11.15

The physio pathological mechanism has not been clarified. It has well known that urinary metabolic abnormalities promoting stone formation are common in patients. Indeed, estrogen may influence the calcium handling, but a link between menopause or the use of menopausal hormones and stones is yet to be made. Hyperinsulinemia, tend to be related to a stronger intestinal absorption as well as renal excretion of calcium. Additionally, insulin resistance may also be associated to urinary acidosis promoting uric acid precipitation. Besides, uric acid stones are more present among overweight and obese patients and in more than 50% among nephrolithiasis women. Since obesity is increasing among women, it could be the link between gender, body fat, insulin level and stone disease [144].

3-6: Calculation of the weight of oxalates by the Titration method.

The volume of KMnO4 contained in the Burette, which appeared in the limits (0.2-8mL), was calculated and the volumes were entered into the following calculations

The size of the patch is 0.45 × 1/2 = the number of milligrams of oxalic acid per 1 mg of stone

Weight of calcium oxalate monohydrate = output × 100 × 1.62

Total calcium weight = weight of calcium oxalate monohydrate × 40÷146.12

Then use these calculations for all samples containing calcium oxalate to calculate their weight. The result was within (2-80 mg) of the weight of 100mg of kidney stones [139].

Figure (3-5) demonstrated a cross-distribution of calculation Calcium weight by titration, stones Calcium weight was increased noticeably in male cases. The range levels of Calcium weight were (1.99- 29.9) mg, while it was (3.99-21.7) mg in the females.

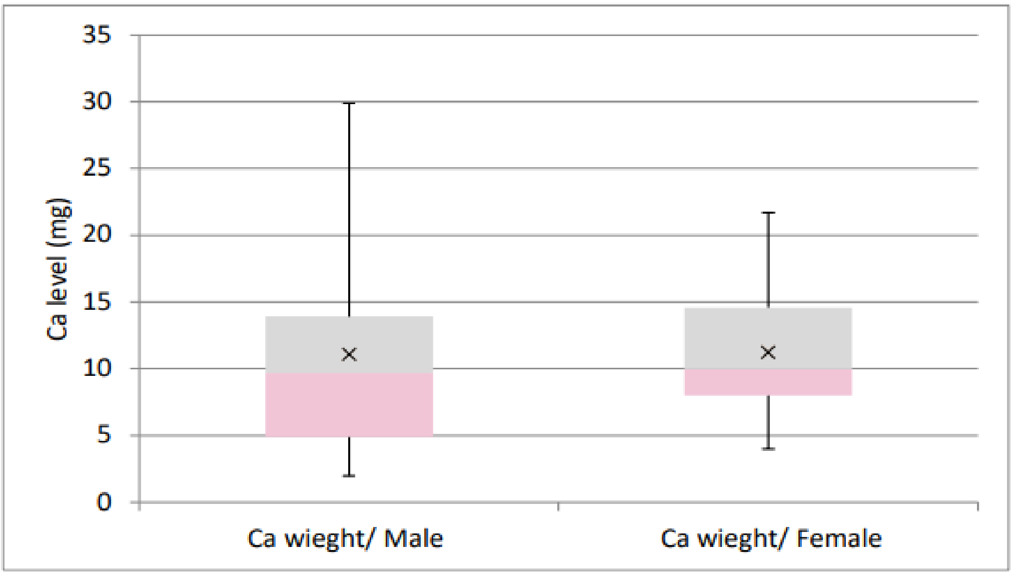

Fig (3-5) the Distribution of Calcium weight in kidney stone patients by titration based on their gender groups

3-7: Estimation of struvite in stones.

($MgNH_4PO_4.6H_2O$) was estimated by estimating magnesium in the pebbles containing it using atomic absorption spectroscopy and then the following equation was used [145].

The results were between (6.85 - 61.22) g / 100g of stone.

Figure (6-3) demonstrated an across distribution of Magnesium levels measured by atomic absorption, stones Magnesium was increased noticeably in male cases. The range levels of Magnesium were (0.75-2.86) ppm in male, while it was (0.38-2.26) ppm in the female group.

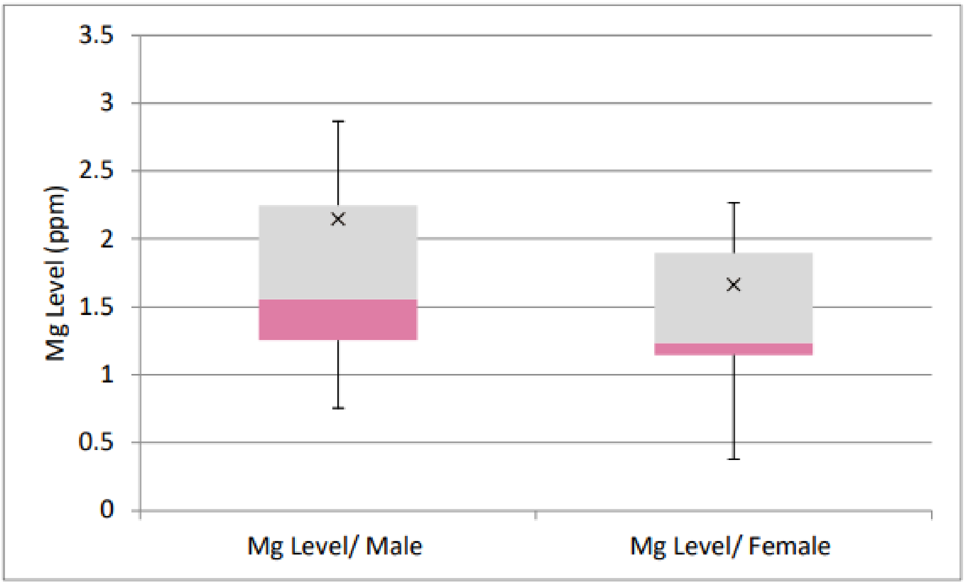

Fig (3-6) Boxplot of the Distribution of Magnesium level in kidney stone patients by atomic absorption based on their gender groups.

Mean differences in Calcium weight and Magnesium levels based on age group

Fig (3-7) shows the difference between calcium weight and magnesium levels based on age groups where a significant increase in stone weight and calcium and magnesium levels were observed in the age group from 32 to 49 years, while they were lower in the age group> 50 years.

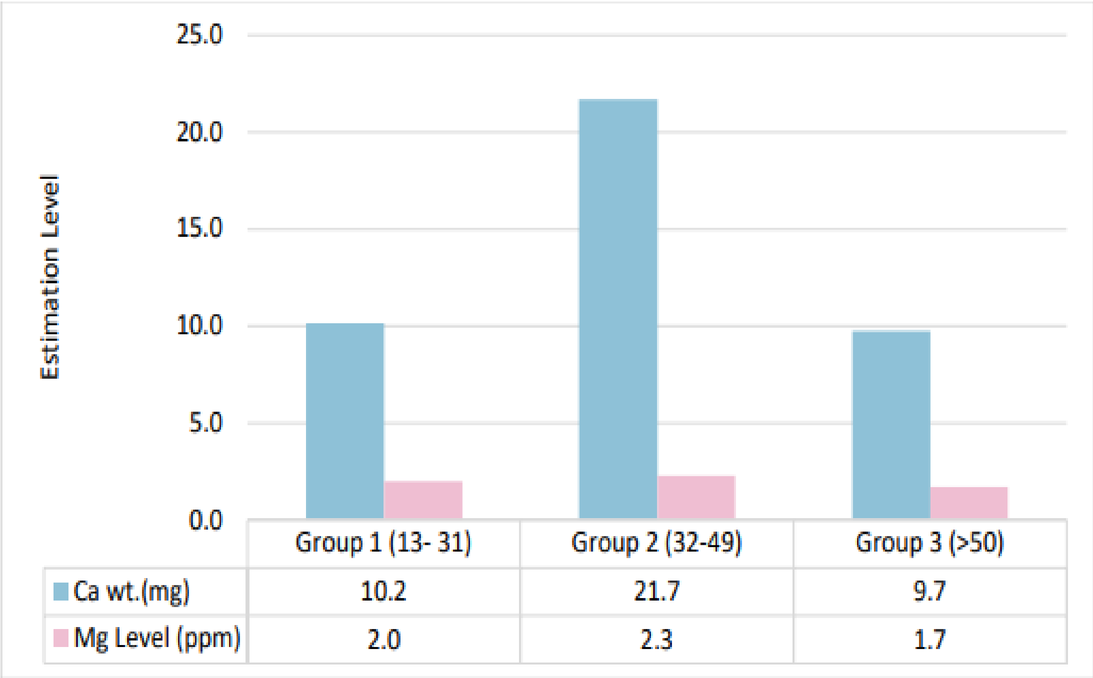

Fig (3-7) The mean differences in Calcium weight and Magnesium levels based on Age groups

3-8: diagnosis of stone components using infrared spectroscopy

The FTIR technique is proposed to be an efficient and accurate technique for assigning the components of urinary stones using infrared spectroscopy and laboratory references to enable us to accurately assign the components of stones. This technique should be developed for all medical centers [146]. Another advantage of this technique, in addition to its speed and accuracy, is the use of small weights of models (10 mg-100). Also, this method together with the X-RAY method gives acceptable and significant analytical results and can be classified as reference methods for the analysis of urinary tract stones.

The infrared absorption spectrum of the study models showed the presence of.

1 -Pure monohydric calcium oxalate in (14) sample Fig (3-8).

The infrared spectrum is characterized by the appearance of absorption beams 1660.71 cm-1 belonging to the stretching C=O group.

The absorption band 989 cm-1 belongs to the bending C-O group.

The absorption band 775.38 cm-1 belong to the C-C Group. [147]

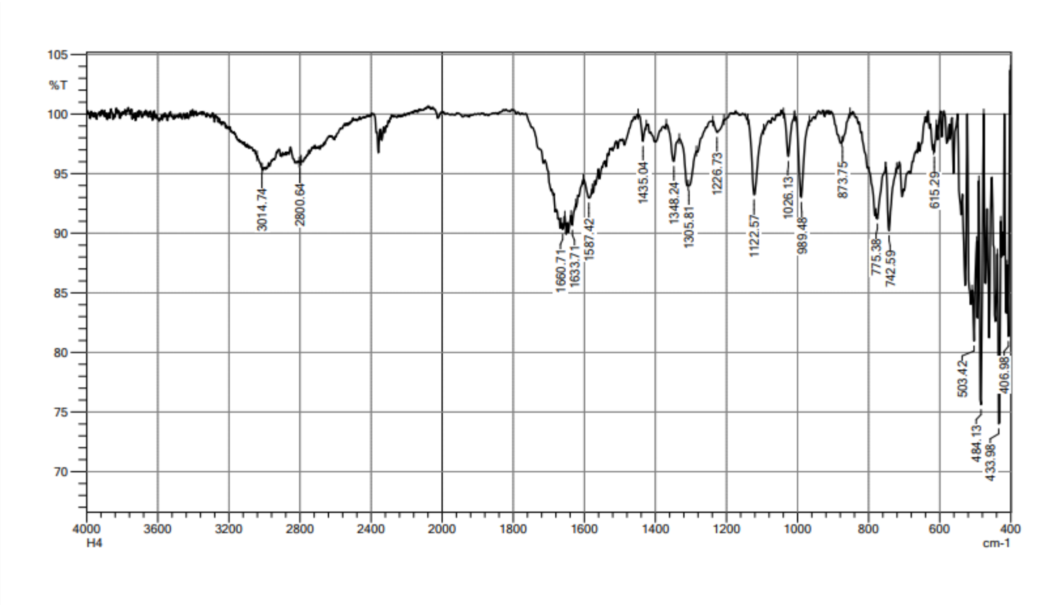

Fig (3-8) Spectrum of pure calcium oxalate monohydrate FTIR

2 - Combination of calcium oxalate monohydrate with calcium phosphate found in one sample Fig (3-9).

The absorption bundle 1620.21 cm-1 belongs to Group C=O.

From calcium oxalate monohydrate and calcium phosphate, the infrared spectrum is distinguished by the appearance of 1028.6 cm-1 absorption beams belonging to the P-O group.

Absorption beams 779.24 cm-1 belong to the C-C Group [148].

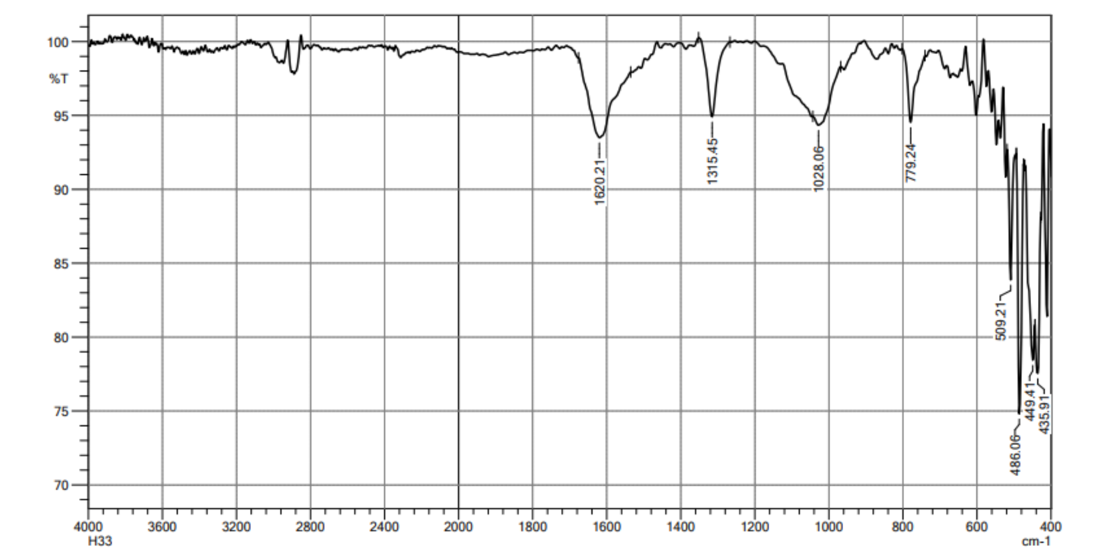

Fig (3-9) FTIR spectrum of the combination of calcium oxalate monohydrate and calcium phosphate

3- Pure uric acid ion appeared in one sample Fig (3-10).

The infrared spectrum is characterized by the appearance of beam 3007 cm-1 belonging to the N-H stretching Group.

The absorption band 1666.05 cm-1 belongs to Group C=O.

The absorption band 1583 cm-1 belongs to C-N bending Group.

The absorption band between 1348 cm-1 belongs to N-H bending Group [149].

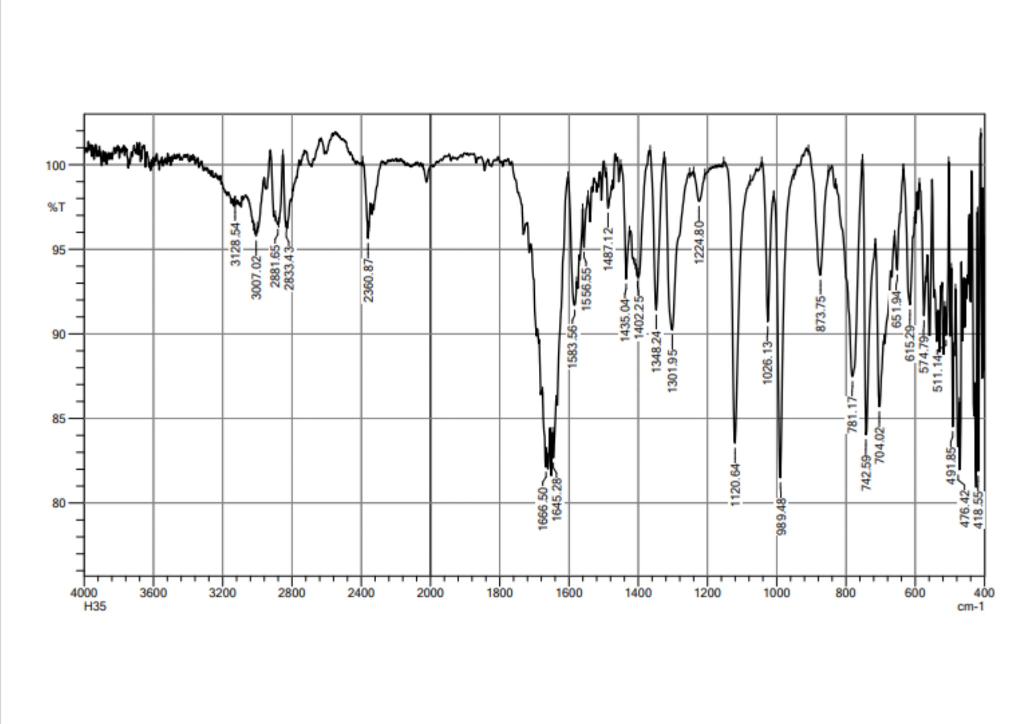

Fig (3-10) FTIR spectrum of pure uric acid

4- A mixture of amino acid and uric acid with calcium oxalate monohydrate in one sample Fig (3-11).

The infrared spectrum is characterized by the appearance of absorption beams of 2920 cm-1 – 2849 cm-1 belonging to the N-H stretching group of the amino acid ion signal.

The absorption beam between 1455 cm-1 - 1436 cm-1 belongs O-H bending group.

The absorption bundle of 1651.07 cm-1 belongs to group C=O.

The absorption beam between 1388 cm-1 - 1385 cm-1 belongs C-N stretching group.

Absorption package 1307.74 cm-1 belongs to the C-O group.

Absorption package 742.59 cm-1 belongs to the C-C group [150].

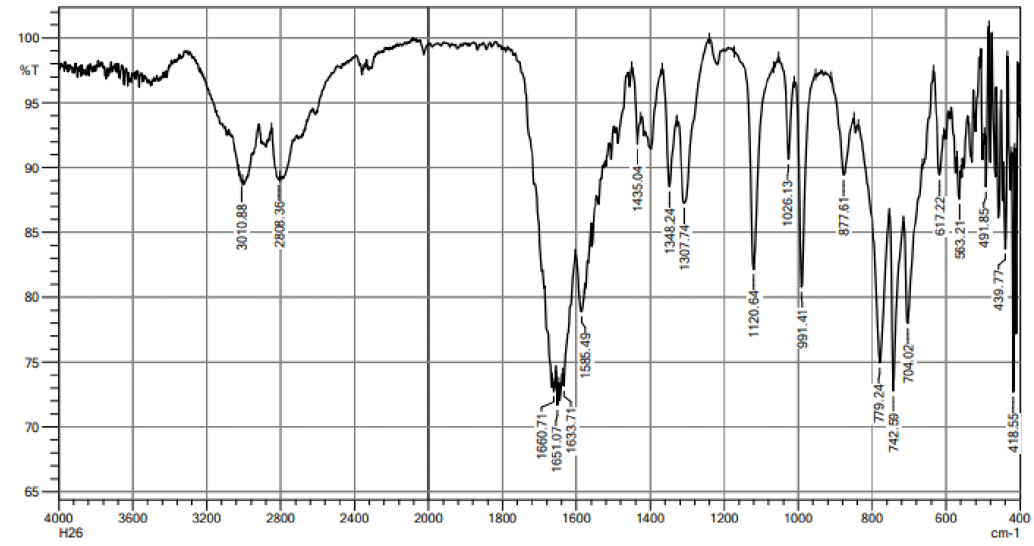

Fig (3-11) FTIR spectrum of the mixture of uric acid with calcium oxalate monohydrate

5 - A mixture of calcium oxalate and magnesium phosphate in (5) sample Fig (3-12).

Struvite stones are easily distinguished in the infrared spectrum by the presence of a peak at 1026.13 cm-1 that shows phosphate ions (PO4-3)

The absorption beam showed between 3010.88 cm-1 - 2821.86 cm-1 belongs to N-H Stretching group.

The absorption bundle of 1716.65 cm-1 belongs to the stretching group C=O.

The absorption bundle of 1311.59 cm-1 belongs to the stretching group

C-O.

The absorption bundle of 877.61 cm-1 belongs to the stretching group

C-C.

And the absorption bundle of 1435cm-1 - 1489 cm-1 belongs to the bending N-H group [151].

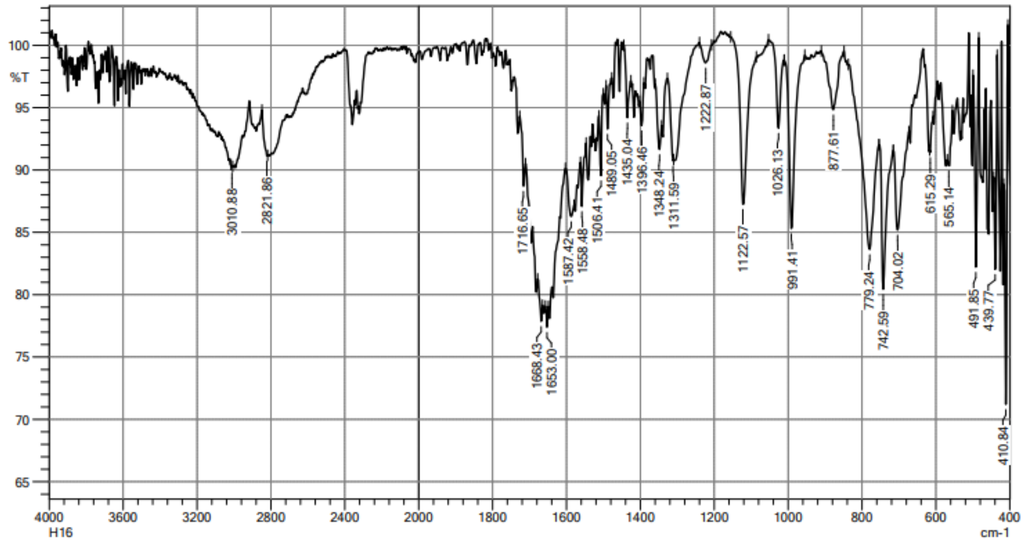

Fig (3-12) FTIR spectrum a mixture of calcium oxalate and magnesium phosphate.

Final conclusion

1- Information on the chemical composition of kidney stones should be considered fundamental in the etiology of diseases.

2- Calcium stones, calcium oxalate monohydrate, and uric acid are among the most common types of stones in patients in Karbala Governorate.

3- Kidney stone disease in men is more common than in women.

4- The highest affected age of kidney stone patients was at >50 years.

Future work

- Many aspects of renal stone formation remain unclear. better understanding of the mechanisms of urolithiasis associated with stone inhibitors or promoters will be critical for stone-removing medications.
- Furthermore, understanding the underlying genetic basis of kidney stone formation will hopefully lead to discover a strategy to manage urolithiasis in the near future.

Chapter Four

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الخلاصة

تهدف هذه الدراسة إلى تحديد المحتوى الكيميائي لحصوات الكلى لدى مرضى الكلى في محافظة كربلاء وتحديد الأماكن الأكثر عرضة لخطر الإصابة بالمرض، وكذلك الجنسين والأعمار الأكثر عرضة لخطر الإصابة بالمرض. تحصي المسالك البولية هو تكوين حصوات في الكلى والمثانة او مجرى البول وهو شائع بشكل متزايد، بمعدل حوالي 12% في جميع أنحاء العالم، ويرتبط بزيادة خطر الإصابة بمرض الكلى في المرحلة النهائية. الشكل الأكثر شيوعًا لحصوات الكلى هو أوكزالات الكالسيوم (Ca Ox) الموجودة على سطح الحليمة الكلوية. يتضمن تكوين الحصى عملية معقدة تنتج عن عدد من المراحل الفيزيائية والكيميائية وهي التشبع، والتنوي، والنمو، والتجميع، واحتباس الحصى داخل الخلايا الأنبوبية. بالإضافة إلى ذلك، تزيد الإصابة الخلوية من احتجاز الجزيئات على السطح الحليمي الكلوي.

أجريت الدراسة باستخدام طرق تحليلية مختلفة مثل الامتصاص الذري ومطيافية الأشعة تحت الحمراء. تم جمع العينات لمرضى حصوات الكلى في مستشفيات محافظة كربلاء بعد اجراء عملية استئصالها في قسم المسالك البولية.

وتم تقسيم الفئات العمرية إلى ثلاث فئات عمرية (13-31) سنة، (32-49) سنة، و(52<) سنة. وكانت حصوات الكلى أكثر شيوعا في المرضى الذين تتراوح أعمارهم بين (52<) سنة. بلغ عدد الذكور 20 (57%) وعدد الإناث 15 (43%). توزعت الحصوات في محافظة كربلاء على حصوات الكالسيوم (80%)، حصوات أوكزالات (57%)، حصوات حمض اليوريك النقي (34%)، خليط حصوات فوسفات الكالسيوم مع حصوات أوكسالات الكالسيوم الأحادية (26%)، وخليط حصوات أوكزالات الكالسيوم أحادية الهيدرات مع حصيات الستروفيت (11%)، بينما السيستين بنسبة (3 %)

وأظهرت النتائج ارتفاع نسبة حصوات الكالسيوم أوكزالات في محافظة كربلاء، حيث كانت أكثر عند الإناث منها عند الذكور. يمكن أن يكون ذلك بسبب العامل الغذائي أو منتجات الألبان وعدم شرب كمية كافية من الماء قد يلعب دورا رئيسيا في تكوين الحصوات.

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كلية التربية للعلوم الصرفة
قسم الكيمياء

تعيين المحتوى الكيميائي لحصى الكلى لمرضى الكلى في محافظة كربلاء

رسالة مقدمة الى
مجلس كلية التربية للعلوم الصرفة – جامعة كربلاء
وهي جزء من متطلبات نيل درجة الماجستير في علوم الكيمياء

كتبت بواسطة

حنين احمد عبد عباس

بكالوريوس كيمياء – جامعة كربلاء (2015)

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