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College of science

A study of Some Biochemical changes in sera patients with kidney stone in Al-Najaf City

A Study Submitted to the College of Science , Karbala University In Partial Fulfillment of The Requirement For The Degree of High Diploma in Chemical Analysis And Drugs

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Dedication

To
my advisors

all my teachers

MY parents

My wife

My brother

My sister

My children

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Haider Naji Kudhair

Abstract

The present study was designed to evaluate biochemical constituents of the sera of patients with renal calculi. Also, this research is designed to evaluate calcium, oxalate and total protein in 24 hour urine for 22 patients compared with 14 other healthy people as control group. The current study deals with the age, sex and smoking effect on the composition of renal calculi and considered to study.

The protocol of the research consisted of qualitative analysis of renal calculi. Twenty-eight calculi were obtained from (8) females and (20) males afflicated with Urolithiasis. Their ages ranged from (7-60) years. Biochemical analysis of sera was carried out by using quantity method analysis and the result was designed to calculate the concentration of Glucose,urea,Creatinine, uric acid, calcium, phosphorus, total protien,acid phosphatase and alkaline phosphatase levels. Showing of resulting data demonstrated that significant $P<(0.05)$ differences of serum glucose concentration between males and females.

The study explained of the effect on biochemical constituent were pointed out that increased significant ($p<0.05$) postive correlation with age for serum glucose, urea, Creatinine ,uric acid, calcium. while appeared decreased significantly ($p>0.05$) negative correlation with age for serum phosphorus, Acid phosphatase and Alkaline phosphatase .

The peak age of patient with renal calculi was found to be in (7-48) years. The effect of smoking factors not to have any changes on the constituents of the study.

Data of urinalysis of 24 hours were increased for concentration calcium, oxalate and total protein of patient when compared with healthy people as control group.

Qualitative assay of renal stone appeared higher percentages uric acid , calcium,oxalate,phosphours and ammonium in calculi of males when compared with that of females.

This study recommendations: Establishment of ideological stone therapy in A L-furat AL-awsat, public educatin,Qualitative analysis of kidney stone ,Using technique (FTIR) ,Control of chronic disease, for example Diabetes mellitus ,Family history ,hypertension, A history of gout,hyperparathyrodisum and Chronic metabolic acidosis. More studies about relation between smoker and renal calculi .

Abbreviations

CaOx	calcium oxalate
CaP	calcium phosphate
UA	Uric acid
CT	Computed tomography
UT	ultrasound
UTIs	urinary tract infections
BMI	Body mass index
GI	gastrointestinal
A	Absorbance
^{o}C	Centigrade
dl	Deciliter
M	Molar concentration
µL	Micro liter
min	minute
EDTA	Ethylene diamine tetra acetic acid
MTB	Methyl thymol Blue
4-AP	4-amino phenazone
DCPS	2,4-dichloro phenol sulfonate
TCA	Tri chloro acetic acid
PVP	poly vinyl pyrrolidone
POD	peroxides
N. S	Non- significant
r	Correlation coefficient
SD	Standard deviation

Chapter One

Introduction and Literature Review

1.1. Introduction:

The existence of kidney stones has been recorded since the beginning of civilization, and lithotomy for the removal of stones is one of the earliest known surgical procedures.[1] In 1901, a stone was discovered in the pelvis of an ancient Egyptian mummy, and was dated to 4,800 BC. Medical texts from ancient, India, China, Persia, Greece and Rome all mentioned calculous disease.[2] New techniques in lithotomy began to emerge starting in 1520, but the operation remained risky.[2],[3]In 1980, Dornier introduced extracorporeal shock wave lithotripsy for breaking up stones via acoustical pulses, and this technique has become into widely use.[4] Kidney stones (renal colic or ureterolithiasis) results from stones or renal calculi (from Latin ren, renes, "kidney" and calculi, "pebbles."[5] in the ureter. The stones are solid concretions or calculi (crystal aggregations) formed in the kidneys from dissolved urinary minerals.

Nephrolithiasis (from Greek nephros, "kidney" (lithos, "stone")) refers to the condition of having kidney stones. Urolithiasis refers to the condition of having calculi in the urinary tract (which also includes the kidneys), which may form or pass into the urinary bladder. Ureterolithiasis is the condition of having a calculus in the ureter, the tube connecting the kidneys and the bladder. The term bladder stones usually applies to Urolithiasis of the bladder.

Kidney stones typically leave the body by passage in the urine stream, and many stones are formed and passed without causing symptoms. If stones grow to sufficient size before passage on the order of at least **(2_3)**millimeters they can cause obstruction of the ureter. The resulting

obstruction causes dilation or stretching of the upper ureter and renal pelvis) as well as muscle spasm of the ureter, trying to move the stone.

This leads to pain, most commonly felt in the flank, lower abdomen and groin (a condition called renal colic). Renal colic can be associated with nausea and vomiting. There can be blood in the urine, visible with the naked eye or under the microscope.[5]

1.2. Kidney stone:

Kidney stones are made of salts and minerals in the urine that stick together to form small "pebbles." They can be as small as grains of sand or as large as golf balls. They may stay in the kidneys or travel out of the body through the urinary tract. The urinary tract is the system that makes urine and carries it out of the body. It is made up of the kidneys, the tubes that connect the kidneys to the bladder (the ureters), the bladder, and the tube that leads from the bladder out of the body (the urethra).When a stone travels through a ureter, it may cause no pain. Or it may cause great pain and other symptoms. [6]

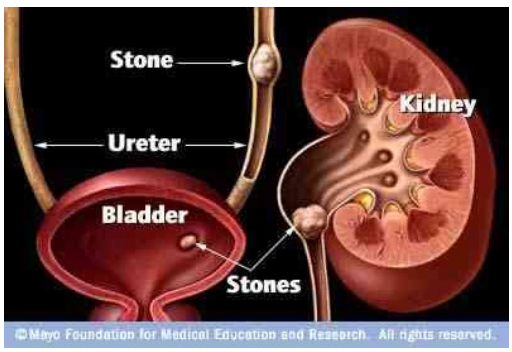


Fig (1-1) kidney stone[1]

1.2.1. Epidemiology:

Within the United States, about 10–15% from adults will be diagnosed with a kidney stone .[7] and the total cost for treating this

condition was US$2 billion in 2003.[8] The incidence rate increases to 20–25% in the Middle East, because of increased risk of dehydration in hot climates. (The typical Arabian diet is also 50% lower in calcium and 250% higher in oxalates compared to Western diets, increasing the net risk.) [9] Recurrence rates are estimated at about 10% per year, totaling 50% over a 5–10 year period and 75% over 20 years. [10]

Men are affected approximately 4 times more often than women.

Recent evidence has shown an increase in pediatric cases. [11]

1.2.2. Mechanism of kidney stone:

1.2.2.1. Supersaturation:

Stones result from a phase change in which dissolved salts condense into solids, and all phase changes are driven by Supersaturation, which is usually approximated for such salts by the ratio of their concentration in the urine to their solubilities. [12] and calculated by computer algorithms.

At Supersaturation values less than 1, crystals of a substance will dissolve; at Supersaturation values greater than 1, crystals can form and grow.

As expected, the composition of stones that patients form correlates with Supersaturation values from the urine they produce.[13] Although increasing urine volume is an obvious way to lower Supersaturation, patients examined in a variety of practice settings have found to be able to increase their urine volume. [14]

Moreover, for unclear reasons, sodium intake and urinary calcium excretion has been found to increase with increased urine volume, partly offsetting the fall in Supersaturation. Along with urine volume, urine calcium and oxalate concentrations are the main determinants of calcium oxalate (CaC_2O_4) Supersaturation; urine calcium concentration and pH

are the main determinants of calcium phosphate (CaP) Super Saturation ;and urinary pH is the main determinant of uric acid (UA) Supersaturation.

1.2.2.2. Crystal Growth and Aggregation:

Homogeneous Nucleation In urine that is supersaturated with respect to calcium oxalate, these two ions form clusters. Most small clusters eventually disperse because the internal forces that hold them together are too weak to overcome the random tendency of ions to move away.

Clusters of over 100 ions can remain stable because attractive forces balance surface losses. Once they are stable, nuclei can grow at levels of Supersaturation below that needed for their creation. The formation product marks the point at which stable nuclei become frequent .enough to create a permanent solid phase

Heterogeneous Nucleation If a supersaturated urine is seeded with preformed nuclei of a crystal that is similar in structure to calcium oxalate, calcium and oxalate ions in solution will bind to the crystal's surface as they would on a seed crystal of calcium oxalate itself. The seeding of a supersaturated solution by foreign nuclei is called heterogeneous nucleation. Cell debris, calcifications on the renal papillae, as well as other urinary crystals, can serve as heterogeneous nuclei that permit calcium oxalate stones to form, even though urine calcium oxalate Supersaturation never exceeds the metastable limit for homogenous nucleation. **(15)**

1.2.3. Types of kidney stone:

There are several types of kidney stones based on the type of crystals of which they consist. The majority are calcium oxalate stones, followed by calcium phosphate stones. More rarely, struvite stones are produced by urea-splitting bacteria in people with urinary tract infections,

and people with certain metabolic abnormalities may produce uric acid stones or cystine stones .[5]

Calcium salts, uric acid, cystine, and struvite ($MgNH_4PO_4$) are the basic constituents of most kidney stones in the western hemisphere. Calcium oxalate and calcium phosphate stones make up 75 to 85% of the total and may be admixed in the same stone. Calcium phosphate in stones is usually hydroxyapatite ($Ca_5(PO_4)_3OH$) or, less commonly, brushite ($CaHPO_4 .H_2O$).[15]

1.2.3.1. Calcium stone:

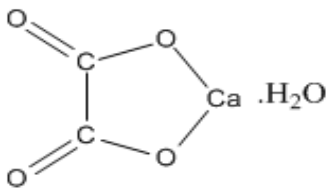
Calcium stones are more common in men; the average age of onset is the third decade. Approximately 60% of people who form a single calcium stone eventually form another within the next 10 years.

The average rate of new stone formation in patients who have had a previous stone is about one stone every 2 or 3 years. Calcium stone disease is frequently familial.

In the urine, calcium oxalate monohydrate crystals usually grow as biconcave ovals that resemble red blood cells in shape and may size but occur in a larger, "dumbbell" form. In polarized light the crystals appear bright against a dark background, with an intensity that is dependent on orientation, a property known as birefringence. Calcium oxalate dihydrate crystals are bipyramidal. Apatite crystals do not exhibit birefringence and appear amorphous because the actual crystals are too small to be resolved by light microscopy.[15]

1.2.3.2.Calcium oxalate stones:

The most common type of kidney stone is composed of calcium oxalate crystals, occurring in about 80% of cases. and the factors that promote the precipitation of crystals in the urine are associated with the development of



calcium oxalate hydrate

$CaC_2O_4.H_2O$

these stones. [10]

(1 -1) structure of calcium oxalate hydrate [1]

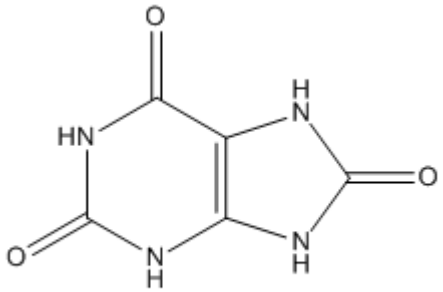
Common sense has long held that consumption of too much calcium could promote the development of calcium kidney stones. However, current evidence suggests that the consumption of low-calcium diets is actually associated with a higher overall risk for the development of kidney stones.[6] This is perhaps related to the role of calcium in binding ingested oxalate in the gastrointestinal tract. As the amount of calcium intake decreases, the amount of oxalate available for absorption into the bloodstream increases; this oxalate is then excreted in greater amounts into the urine by the kidneys. In the urine, oxalate is a very strong promoter of calcium oxalate precipitation, about 15 times stronger than calcium. The formation of calcium phosphate stones is associated with conditions such as hyperparathyroidism and renal tubular acidosis. [16]

1.2.3.3. Non Calcium stone:

:1.2.3.4. Uric acid stone

Uric acid stones are more prevalent in men than females. Half of patients with uric acid stones have gout. In urine, uric acid crystals are red-orange in color because they absorb the pigment uricine. Anhydrous uric acid produces small crystals that appear amorphous by light microscopy. Uric acid dihydrate tends to form teardrop-shaped crystals as well as flat, rhomboid plates; both are strongly birefringent. Uric acid

gravel appears like red dust, and the stones are also orange or red on some occasions. [15]



URIC ACID

(1 - 2) structure of uric acid [2]

About 5–10% of all urinary stones are formed from uric acid. [10] Uric acid

Stones form in association with conditions that cause hyperuricosuria with

or without high blood serum uric acid levels (hyperuricemia), and with Acid/base metabolism disorders where the urine is excessively acidic (low

$_{P}$H) resulting in uric acid precipitation. A diagnosis of uric acid Nephrolithiasis is supported if there is a radiolucent stone, a persistent

Undue urine acidity, and uric acid crystals in fresh urine samples.[16]

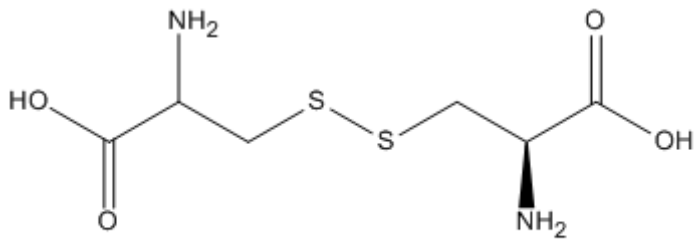
1.2.3.5. Struvite stone:

Struvite stones are common and potentially dangerous. These stones occur mainly in women or patients who require chronic bladder catheterization and result from urinary tract infection with urease-producing bacteria, usually Proteus species. The stones can grow to a large size and fill the renal pelvis and calyces to produce a "staghorn" appearance.

They are radiopaque and have a variable internal density. In urine, struvite crystals are rectangular prisms said to resemble coffin lids· [15] The formation of struvite stones is associated with the presence of urea-splitting bacteria, most commonly Proteus mirabilis (but also Klebsiella, Serratia, Providencia species). These organisms are capable of splitting urea into ammonia, decreasing the acidity of the urine and resulting in favorable conditions for the formation of struvite stones. Struvite stones are always associated with urinary tract infections. [16]

1.2.3.6. Other stones:

Formation of cystine stones is uniquely associated with people suffering from cystinuria, who accumulate cystine in their urine. Cystinuria can be caused by Fanconi's syndrome .[16]



CYSTINE

(1-3) structure of cystine[3]

1.2.4. Causes of kidney stone:

Kidney stones can be due to underlying metabolic conditions, such as renal tubular acidosis.[10]Dental disease. [17] hyperparathyroidism.[18] and medullary sponge kidney.[19] Patients with recurrent kidney stones should be screened for these disorders. This is typically done with a 24 hour urine collection that is chemically analyzed for deficiencies and excesses that promote stone formation. Kidney stones are also more common in patients with Crohn's disease. [20]

There has been some evidence that water fluoridation may increase the risk of kidney stone formation. In one study, patients with symptoms of skeletal fluorosis were 4.6 times as likely to develop kidney stones.[21] However, fluoride may also be an inhibitor of urinary stone formation.[22] A 1998 paper in the Archives of Internal Medicine examined the sources of a widely-held belief in the medical community that vitamin C can cause kidney stones.[23] The American Urological Association has projected that increasing global temperatures will lead to greater future prevalence of kidney stones.[24]

Kidney stones may form when the normal balance of water, salts, minerals, and other substances found in urine changes. How this balance changes determines the type of kidney stone. Most kidney stones are calcium-type-they form when the calcium levels in urine change. Factors that change urine balance include:

(1)-Not drinking enough water:

Try to drink enough water to keep the urine clear (2.0-2.5 L). When insufficient fluid in take. the salts, minerals, and other substances in the urine can stick together and form a stone. This is the most common cause of kidney stones.

(2)-Medical conditions:

Many medical conditions can affect the normal balance and cause stones to form. Gout is one example. Also, people who have inflammatory bowel disease or who have had surgery on their intestines may not absorb fat from their intestines in a normal way. This changes the way the intestines process calcium and other minerals, and it may lead to kidney stones.

More commonly, kidney stones can run in families, as stones often occur in family members over several generations.

In rare cases, a person forms kidney stones because the glands produce too much of a hormone, which leads to higher calcium levels and possibly calcium kidney stones. [6]

1.2.5. Risk factors and recurrent stone formation: [25]

Several factors make it more likely will get kidney stones. Some of These can control, and others cannot.

1.2.5.1. Risk factors can control (Risk factors): [25]

Risk factors for both new and recurring kidney stones that can control include:

1- Fluids drink. The most common cause of kidney stones is insufficient fluid in take. Try to drink enough water to keep urine clear (2-2.5 L).Drinking grapefruit juice may increase risk for recurrence kidney stone.

2- Dietary factor , if we that diet may be a problem, schedule an appointment with a dietitian and review food choices.

3- Vitamins C and D can increase risk of kidney stones when take more than the daily recommendations. and do not take more than the recommended daily doses. and vitamins A deficiency .

4- Levels of calcium affect greater risk of kidney stones. Getting on recommended amounts of calcium combined with a low-sodium, low-protein diet may decrease risk of kidney stones.

5- Diets high in protein, sodium, and oxalate-rich foods, such as dark green vegetables, increase greater risk for developing kidney stones.

6- Weight gain can result in both insulin resistance and increased calcium in the urine, which can result in a greater risk for kidney stones. In one study, weight gain since early adulthood, a high body mass index (BMI), and a large waist size increased a person's risk for kidney stones.

7- Activity level. People who are not very active may have more problems with kidney stones.

8- Medicine: Some medicines, such as acetazolamide (Diamox), indinavir (Crixivan), antacids and aspirin can cause kidney stones to form.

1.2.5.2 Risk factors cannot control (Risk marker): [25]

Risk factors for both new and recurring kidney stones that cannot control include:

1- Age and gender. Men between the ages of 30 and 50 are most likely to get kidney stones.Postmenopausal women with low estrogen levels have an increased risk for developing kidney stones. Women who have had their ovaries removed are also at increased risk.

2- A family history of kidney stones. Congenital or inherited causes .

3- Other diseases or conditions, such as inflammatory bowel disease, cystic fibrosis, gout, or hypertension. Insulin, which can occur because of diabetes or obesity or Prolonged bed rest.

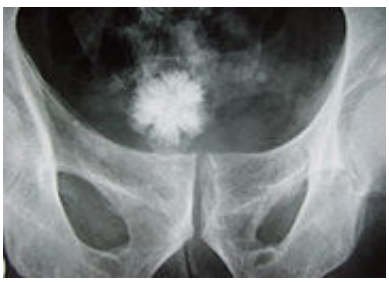
4- Bladder problems caused by spinal cord injury.

5-. Abnormal urinary tract, such as the kidneys being joined (horseshoe kidneys).

1.2.6. Diagnosis of kidney stones:

Clinical diagnosis is usually made on the basis of the location and severity of the pain, which is typically colicky in nature (comes and goes in spasmodic waves). Pain in the back occurs when calculi produce an obstruction in the kidney. [26]

Imaging is used to confirm the diagnosis and a number of other tests can be undertaken to help establish both the possible cause and consequences of the stone.



Fig(1-2) star-shaped bladder urolith on an x-ray of the pelvis.(2)

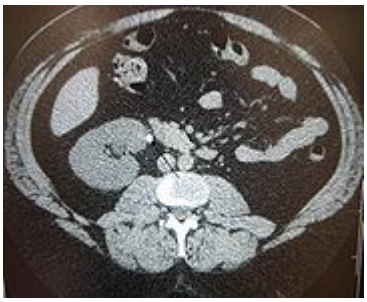
A –x rays

The relatively dense calcium renders these stones radio-opaque and they can be detected by a traditional X-ray of the abdomen that includes the Kidneys, Ureters and Bladder—KUB.[27] This may be followed by an IVP (Intravenous Pyelogram); which requires about 50 ml of a special dye to be injected into the bloodstream that is excreted by the kidneys and by its density helps outline any stone on a repeated X-ray. These can also be detected by a Retrograde pyelogram where similar "dye" is injected directly into the ureteral opening in the bladder by a surgeon.

b-Computed tomography:

Computed tomography without contrast is considered the gold-standard diagnostic test for the detection of kidney stones. All stones are detectable by CT except very rare stones composed of certain drug residues in the urine.[27]

If positive for stones, a single standard x-ray of the abdomen (KUB) is recommended. This gives a clearer idea of the exact size and shape of the stone as well as its surgical orientation. Further, it makes it simple to follow the progress of the stone by doing another x-ray in the future.



1. Fig(1-3) Right side kidney stone with mild hydronephrosis(3)

C- Ultrasound:

Ultrasound imaging is useful as it gives details about the presence of hydronephrosis.[27] It can also be used to detect stones during pregnancy when x-rays or computed tomography(CT) are discouraged. Radiolucent stones may show up on ultrasound however they are also typically seen on computed tomography scans. Some recommend that US be used as the primary diagnostic technique with computed tomography (CT) being reserved for those with negative Ultrasound(US) result and continued suspicion of a kidney stone. This is due to its lesser cost and avoidance of radiation.[28]

d-Other methods:

Other investigations typically carried out include: [27,29,30]

(1)- Microscopic study of urine, which may show proteins, red blood cells, bacteria, cellular casts and crystals. Culture of a urine sample to exclude urine infection (either as a differential cause of the patient's pain, or secondary to the presence of a stone.

(2)- Blood tests, Full blood count for the presence of a raised white cell count (Neutrophilia) suggestive of infection, a check of renal function and to look for abnormally high blood calcium levels (hypercalcaemia).

(3)- 24 hour urine collection to measure total daily urinary volume, magnesium, sodium, uric acid, calcium, citrate, oxalate and phosphate.

1.2.7. Kidney stones- medications:

1.2.7. 1. Prevention of kidney stones:

Preventive strategies include dietary modifications and sometimes also taking drugs with the goal of reducing excretory load on the kidneys [6,31].

(1)- Drinking enough water to make 2 to 2.5 liters of urine per day to produce a large amount of urine. Some people might need to get fluids through a vein (intravenous).

(2)- A diet low in protein, nitrogen and sodium intake. Restriction of oxalate-rich foods, such as chocolate, nuts, soybeans.[32] rhubarb and spinach, plus maintenance of an adequate intake of dietary calcium. There is equivocal evidence that calcium supplements increase the risk of stone formation, though calcium citrate appears to carry the lowest, if any, risk.

Taking drugs such as thiazides, potassium citrate, magnesium citrate and allopurinol, depending on the cause of stone formation. Some fruit juices, such as orange, blackcurrant, and cranberry, may be useful for lowering the risk factors for specific types of stones. Orange juice may help prevent calcium oxalate stone formation, black currant may help prevent uric acid stones, and cranberry may help with UTI-caused stones.[33,34] Avoidance of cola beverages.[35,36] Avoiding large doses of vitamin C.[37]

For those patients interested in optimizing their kidney stone prevention options, a 24 hour urine test can be a useful diagnostic.

Restricting oxalate consumption, Calcium plays a vital role in body chemistry so limiting calcium may be unhealthy. Since calcium in the intestinal tract will bind with available oxalate, thereby preventing its absorption into the blood stream, some nephrologists and urologists recommend chewing calcium tablets during meals containing oxalate foods. This is only helpful in those patients who are absorbing excess oxalate which is a minority of patients as most oxalate excreted in the urine is actually made by the liver.

(3)- Decreased protein diet**:**

A high protein diet might be partially to blame. Protein from meat and other animal products is broken down into acids, including uric acid. The most available alkaline base to balance the acid from protein is calcium phosphate (hydroxyapatite) from the bones (buffering). The kidney filters the liberated calcium which may then form insoluble crystals (i.e., stones) in urine with available oxalate (partly from metabolic processes, partly from diet) or phosphate ions, depending on conditions. High protein intake is therefore associated with decreased bone density as well as stones. The acid load is associated with decreased

urinary citrate excretion, citrate competes with oxalate for calcium and can thereby prevent stones. [38]

(4)- Other modifications:

Potassium citrate is also used in kidney stone prevention. This is available as both a tablet and liquid preparation. The medication increases urinary pH (makes it more alkaline), as well as increases the urinary citrate level, which helps reduce calcium oxalate crystal aggregation. Though caffeine does acutely increase urinary calcium excretion, several independent epidemiologic studies have shown that coffee intake overall is protective against the formation of stones.[38]

1.2.7.2. Treatment:

The goal of treatment is to relieve symptoms and prevent further symptoms. (Kidney stones that are small enough usually pass on their own.) Treatment varies depending on the type of stone. People with severe symptoms might need to be hospitalized.

Collect urine for 24 hours after pass a stone, so can check urine to help determine the type and cause of the stone. Knowing the type of the stone may help prevent getting stones in the future. Most small stones [less than 5mm] move out of the body (pass) without the need for any treatment other than taking pain medicine and drinking enough fluids. The smaller a stone is, the more likely it is to pass on its own. About 9 out of every 10 stones smaller than 5mm and about 5 out of every 10 stones 5mm to 10mm pass on their own. [6]

The average time a stone takes to pass ranges between 1 and 3 weeks, and two-thirds of stones that pass on their own pass within 4 weeks of when the symptoms appeared.[39]

If pain is too severe, the stones are blocking the urinary tract or if also have an infection, there is probably suggest medical or surgical treatment that include other treatment are:

(1)-Extracorporeal shock wave lithotripsy (ESWL) . [40]

ESWL uses shock waves that pass easily through the body but are strong enough to break up a kidney stone. This is the most commonly used medical treatment for kidney stones.

(2)-Ureteroscopy. The surgeon passes a very thin telescope tube (ureteroscope) up the urinary tract to the stone's location, where uses instruments to remove the stone or break it up for easier removal. Occasionally, may need a small hollow tube (ureteral stent) placed in the ureter for a short time to keep it open and drain urine and any stone pieces. Ureteroscopy is often used for stones that have moved from the kidney to the ureter.

(3)- Percutaneous nephrolithotomy or nephrolithotripsy.

The surgeon puts a narrow telescope into the kidney through a cut in back of kidney then removes the stone (lithotomy) or breaks it up and removes it (lithotripsy).This procedure may be used if ESWL does not work or if have a very large stone.

(4)- Open surgery.

The surgeon makes a cut in the side or the belly to reach the kidneys and remove the stone. This treatment is rarely used. The size of the stone, its location in the urinary tract, overall health, and other factors

are all considered in deciding which method to use when breaking up or removing a kidney stone.

1.2.7.2.1 . Medicine for the treatment of calcium stones: [6]

About 80% of kidney stones are calcium stones. Calcium stones cannot be dissolved by changing diet or taking medicines. These medicines may keep calcium stones from getting bigger or may prevent new calcium stones from forming. Thiazides (such as hydrochlorothiazide, chlorthalidone) and potassium citrate are commonly used to prevent calcium stones. Orthophosphate is sometimes used. It has more side effects than thiazides or potassium citrate.

1.2.7.2.2 . Medicine for the treatment of uric acid stones: [6]

About 5% to 10% of kidney stones are made of uric acid, a waste product that normally exits the body in the urine.

Uric acid stones can sometimes be dissolved with medicine. Potassium and sodium bicarbonate (baking soda) prevent the urine from becoming too acidic, which helps prevent uric acid stones.Allopurinol (Lopurin, Zyloprim) makes it more difficult for body to make uric acid.

1.2.7.2.3. Medicine for the treatment of cystine stones: [6]

Less than 1% of kidney stones are made of a chemical called cystine. Cystine stones are more likely to occur in families with a disease that results in too much cystine in the urine (cystinuria).Potassium citrate prevents the urine from becoming too acidic, which helps prevent cystine kidney stones from forming. Penicillamine (Cuprimine, Depen), tiopronin, and captopril (Capoten) all help keep cystine dissolved in the urine, which makes cystine-type kidney stones less likely to form.

1.2.7.2.4. Medicine for the treatment of struvite stones: [6]

About 10% to 15% of kidney stones are struvite stones. They can also be called infection stones if they occur with kidney or urinary tract infections (UTIs).

These type of kidney stones sometimes are also called staghorn calculi if they grow large enough.Acetohydroxamicacid,Urease inhibitors (Lithostat) are rarely used because of their side effects and poor results.

1.2.7.2.5. Diuretics:

One of the recognized medical therapies for treatment of stones is:

(A)- Thiazides: [6]

a class of drugs usually thought of as diuretics. These drugs prevent calcium stones through an effect independent of their diuretic properties: they reduce urinary calcium excretion. Sodium restriction is necessary for clinical effect of thiazides, as sodium excess promotes calcium excretion.

Thiazides work best for renal leak hypercalciuria a condition in which the high urinary calcium levels are from a primary kidney defect. They work well initially for absorptive hypercalciuria . a condition in which high urinary calcium is a result of excess absorption from the GI tract. With this condition they lose effectiveness over time, typically around 2 years, and patients need a period off treatment to regain effectiveness.Thiazides will cause hypokalemia and reduced urinary citrate levels so should be given with supplements for each, usually as a potassium citrate preparation.

(B)-Allopurinol:

Allopurinol (Zyloprim) is another drug with proven benefits in some calcium kidney stone formers. Allopurinol interferes with the liver's

production of uric acid.Hyperuricosuria, too much uric acid in the urine, is a risk factor for calcium stones. Allopurinol reduces calcium stone formation in such patients. The drug is also used in patients with gout or hyperuricemia.[41]

However, hyperuricemia is not the critical feature of uric acid stones, which can occur in the presence of hypouricemia. Uric acid stones are more often caused by a combination of high urine uric acid and low urine pH.

Even relatively high uric acid excretion will not be associated with uric acid stone formation if the urine pH is alkaline. Therefore prevention of uric acid stones relies on alkalinization of the urine with citrate (sodium citrate, sodium bicarbonate, potassium citrate, potassium bicarbonate or acetazolamide, a carbonic anhydrase inhibitor.Allopurinol is reserved for patients in whom alkalinization is difficult. For patients with increased uric acid levels and calcium stones, Dosage is adjusted to maintain a reduced urinary excretion of uric acid. Serum uric acid level at or below 6 mg/dL is often the goal of the drug's use in patients with gout or hyperuricemia. [42]

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1.2.8. Aims of the study

The aimes of the study are:

1- To evaluate compositions of kidney stone quality .

2- To clarify the influence of sex, age and smoking on the kidney stone composition .

3- Investigation on the change in the level of some enzymes in serum of patients with kidney stone .

4- To predict the relation between kidney stone and change to some biochemical (parameter) .

Chapter Two

Material and Methodes

Materials and methods

2.1. Materials

The materials are listed in table (2-1) and (2-2) .

2.1.1. laboratory Instruments

Table (2-1):list of Instruments used .

Instrument	company	source
Centrifuge	Kubota	Japan
Spectrophotometer	Cecil Ce 1011	France
Water bath	Memmert	Germany

2.1.2Chemicals:

The chemicals and kits that were used in this study were of the highest purity and are listed in the table below with their suppliers .

Table (2-2) chemicals with their suppliers .

Material	Company	Purity%
glucose kit	spin react	-
Urea kit	biomerieux	-
Creatinine kit	syrbio	-
Uric acid kit	spin react	-
Calcium kit	biomerieux	-
Inorganic phosphorous kit	randox	-
Alkaline phosphates kit	biomerieux	-
Acid phosphates kit	Human	-
Total protien kit	spin react	-
Commercial kit of stone analysis.	Vaccine and sera institute Iraq _ Baghdad (VSI).	-
Nitric acid	Laboratory Reagent,(Gain land chemical company)GCC.	69-71%

Hydrochloride acid	Fluka,swizerland	98%
Sulfuric acid	Carlo Erba	95%
Sodium hydroxide	Merck , Germany	98%
Potassium permanganate	Merck , Germany	97%
Mercuric iodide	BDH,U.K	98%
Potassium iodide	Merck, Germany	98%

2.1.3. Patients and control subjects:

During the period from July/2009 to December /2009 **.**Twenty eight stones obtained from patients afflicted with urolithiasis.They were 20 males and 8 females. Their ages ranged from (7_60) years.

All of them attended the hospital of Al-Sadder teaching in Najaf city. Stones were obtained by surgery .

The stones were washed to remove any dried blood or other matter, Dried in an incubator at 37 ^{o}C, And weight .

They were pulverized in a motor ,a sample of the powered stone was weighted. The residue after drying was divided into aliquots for chemical analysis.The stone were subjected to quantitative analysis to determine composition

2.1.3.1 Control Group:

The control group consists of people who were collected from medical staff and relative who were free from signs and symptoms of Renal disease, liver disease , cardiac disease, diabetes mellitus and hypertension in AL-Sadder teaching hospital in Najaf city They were 8 females and 20 males

2.1.3.2 Collection of Samples:

Five milliliters of venous blood were drawn from each fasting patient (8-12 hours fasting). Slow aspiration of the venous blood sample via the

needle of syringe to prevent Hemolysis with tourniquet applied else fasting blood collection to purpose electrolytes analysis above the anterior cubital fossa . All the samples that were grossly Hemolysed were neglected and other new samples were taken .

The collection of samples was conducted for a period of five months ,starting from (July (2009) ending (December (2009) The samples were dropped into clean disposable tubes ,left at room temperature for 20 minutes for clot formation and then centrifuged for 15 minutes at 3500 per minute.

2.2 Methods:

2.2.1 Determination of Inorganic phosphorous Concentration:

principle:

inorganic phosphate in serum reacts with molybdic acid to form a phosphor molydic acid complex, which is reduced by ammonium iron(Π)sulphate to molybdenum blue ,which is measured at 690nm. **(43).**

Procedure:

Table (2-3): procedure for determination inorganic phosphorous in serum .

	Reagent blank	Standard	Sample
Working reagent	1.0(mL)	1.0(mL)	1.0(mL)
Deionized water	30(µL)	-	-
Standard	-	30(µL)	-
Sample	-	-	30(µL)

The Mixtured and then let stand for 10 min at 25˚C. Read absorbance of sample(A sample) and standard (A standard) against reagent blank,which is measured at 690 nm.

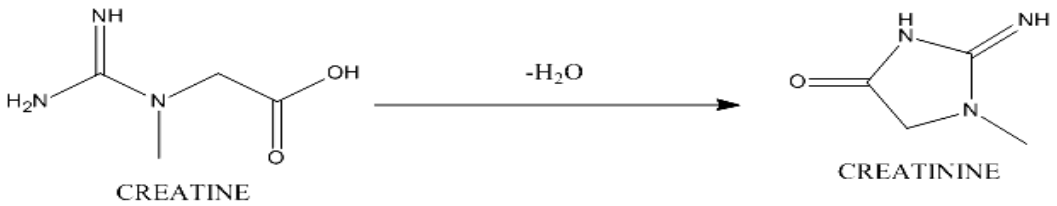
Calculation:

concentration of inorganic phosphorous

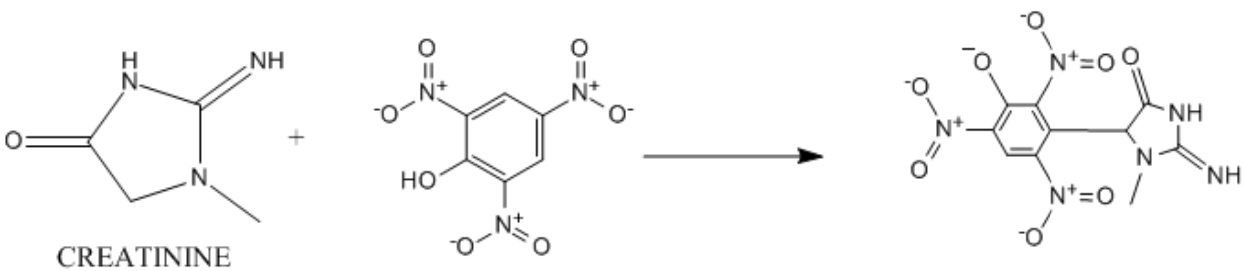
$$\frac{\text{A sample}}{\text{A standard}} \times \text{concentration of standard.}$$

2.2.2 Determination of Creatinine concentration:

Principle: Creatinine in alkaline picrate solution, forms a color complex (44).



CREATINE CREATININE



CREATININE PICRIC ACID Red creatinine -picreate complex

Reagents:

Sodium hydroxide(R1)	1.6 mol /l

Picric acid **(**R2**)**	35 m mol/l
Standard (R3)	2.0 mol/l

Reagents are stable at room temperature.

Additional reagent:

Tri chloro acetic acid (TCA) 10%

Preparation of working reagent:

Mixed proportionally 1/1 the reagent R1 and R2

Deproteinisation procedure

Tri color acetic acid	0.5 ml
Serum or plasma	0.5 ml

Mixtured well. Centrifuge at 3500 rpm for 10 min then pour off the supernatant.

Procedure:

Table (2-4): Procedure for determination of Creatinine concentration in serum

	Blank	Standard	Sample
distilled water	0.5(mL)		-
Trichloroacetic acid solution	0.5(mL)	0.5(mL)	-
Standard	-	0.5(mL)	-
supernatant	-	-	1.0(mL)
Reagent mixture	1.0(mL)	1.0(mL)	1.0(mL)

Mixtured and then let stand for 20 min at room temperature, read absorbance of sample(A) and standard (A standard) against reagent blank. which is measurement in 550 nm at 25˚C**.**

Calculation:

Concentration of Creatinine in serum or plasma:

$$\frac{\text{(A) sample}}{\text{(A) standard}} \times \text{concentration of standard.}$$

2.2.3. Determination of calcium Concentration in serum:

Principle:

Ca-kit enables colorimetric determination of total calcium without deproteinization, in human urine and serum. The calcium ion react with the methylthymol blue indicator(MTB) in alkaline medium**.**

$$\mathbf{Ca^{++} + MTB \longrightarrow Ca\text{-}MTB\ complex}$$

The color intensity of the Ca-MTBcomplex, measured at 612nm ,is proportional to the quantity of calcium present in the sample.8-hydroxyquinoline eliminations interference from magnesium.

Poly vinyl pyrrolidone(PVP) eliminates interference from proteins**.** **(45)**

Procedure:

Table (2-5) : Procedure for determination of calcium concentration in serum

	Reagent blank	standard	sample
standard	_	10 µL	_
sample	_	_	10 µL
	0.5ml	0.5ml	0.5ml
	0.5ml	0.5ml	0.5ml

Reagent(2):(methylthymolblue8-hydroxyquinoline,PVP.Reagent(3):mono ethanol amine (MEA).

Mixtured and then performed photometry after one minute at 612nm and 25˚C.

Calculation:

$$\text{sample concentration} = \frac{\text{(A) sample}}{\text{(A) standard}} \times \text{concentration of standard}$$

2.2.4 Determination of acid phosphates Concentration:

Principle:

1-naphthyl phosphate is hydrolysed by acid phosphatase(AC-P) to phosphate and 1-naphthol,which is converted (1-naphthyl phosphate) to an azo dye.

the increase of absorbance at 405nm is proportional to the total acid phosphtase activity in the sample. The prostatic acid phosphatase can be determined indirectly by calculation of activity difference. **(46)**

Reaction principle:

1-naphthyl phosphate+H_2O ⟶ phosphate +1-naphthol

1-naphthol + 1-naphthyl phosphate ⟶ azo dye

Procedure: Warm working reagent and cuvettes up to the desired temperture (25˚C)temperture must be kept constant for the duration of the test. pipette directly into Ra+Rb containing working reagents. Mixed and

transferred the solution into acuvette Ra and Rb.which Ra(Total acid phosphate)+Rb(prostastic acid phosphatase).

Table (2-6) : Procedure for determination of acid phosphates in serum

Pipette into cuvettes		
sample	100 µL	100 µL
Working reagent Ra	1000 µL	_
Working reagent Rb	_	1000 µL

Ra: citrate buffer($_p$H 5.2),Rb:1-naphtyl phosphate).

Mixtured,readed the absorbance A1 after 5 minutes and start the stop watch at the same time and wave length at 405 nm**.** Readed the absorbance A2 exactly after5min.at 25˚C.

A2-A1= Δ **A**

Calculation:

Calculation the total acid phosphatase activity in sample using following factor**:**

U/I	25˚C
Total acid phosphatase (ΔA reagent Ra) x	149

2.2.5 Determination of alkaline phosphatase Concentration:

Principle:

Colorimetric determination of alkaline phosphatase activity according to the following reaction :

Phenyl phosphate $\xrightarrow[\text{PH 10}]{\text{Alkaline phosphates}}$ **phenol+phosphate**

The liberated phenol is measured in presence of 4-aminoantipyrine and potassium ferricyanid.the presence of sodium arsenate in the reagent stop the enzymatic reaction[(47).]

procedure:

Table (2-7) : Procedure for determination of alkaline phosphates in serum

	Serum sample	Serum blank	standard	Reagent blank
Reagent(1)	2 ml	2 ml	2 ml	2 ml

Reagent(1): (disodium phenyl phosphate bicarbonate, buffer pH=10,sodium merthiolate

Incubated for exactly 15 minutes at 37 ^{o}C.

Serum	٥٠ µL	_	_	_
Reagent(2)phenol	_	_	٥٠ µL	_

Incubated for exactly 15 minutes at 37 ^{o}C.

Reagent(3)	0.5ml	0.5ml	0.5ml	0.5ml

Reagent(3): 4-amino antipyrine,sodiumarsenate. Mixtured potassium ferricyanid.

Distilled water well or preferably vortex.

Reagent(4)	0.5ml	0.5ml	0.5ml	0.5ml
serum	_	٥٠ µL	_	_
Distilled water	_	_	_	٥٠ µL

Mixtured and then let stand for 10 minutes in the dark measure and wave length is 510nm.

Calculation: (A) serum sample – (A) serum blank

sample concentration = ——————————— x concentrate stander

(A) standard

U/100ml: n=20
U/L: n=142

2.2.6. Determination of uric acid Concentration:

Principle:

Uric acid is oxidized by uricase to all Antoine and peroxide(H_2O_2), which under the influence of (4-AP) (4-aminophenazone) and(DCPS) (2,4-di chlorophenol sulfonate forms a red quinoneimine compound:

Uric acid $+2H_2O+O_2$ —Uri case→ Allantoine + CO_2 + $2H_2O_2$

$2H_2O_2$+ 4-AP+DCPS —POD→ quinoneimine+$4H_2O$

the intensity of the red color formed is proportional to the uric acid concentration in the sample. **(48),(49).**

Procedure:

(1) adjust the instrument to zero with distilled water.

(2) Pipette into acuvette.

Table (2-8): Procedure for determination of uric acid concentration in serum

	blank	Standard	Sample
Working reagent(ml)	1.0	1.0	1.0
Standard (µL)	_	25	_
sample(µL)	_	_	25

(3) mixtured and incubate for 10min at (25 ^{o}C).

(4)-Readed the absorbance (A) of the samples and standard , against the blank at wavelength 520 nm. The color is stable for at least 30 minutes.

Calculation of concentration of uric acid:

$$\frac{\text{A sample}}{\text{A standard}} \times \text{concentration of standard}$$

2.2.7. Determination of urea Concentration:

urea kits enable end enzymatic determination of urea concentration(urease_modified bertha lot reaction in human) serum, plasma urease hydrolyzes urea by producing ammonium**.**

$$\text{UREA} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

In an alkaline medium, the ammonium ions react with the salicylate and hydrochlorite to form agreen colored indophenol(2,2_dicarboxylindophenol).the reaction is catalyzed by the sodium nitroprusside**.**

NH_4+ salicylate+hydrochorid ⟶ indophenol.

The color intensity is proportional to the urea concentration in the sample .the possible presence of heavy of metals inhibits the formation of indophenol EDTA lifts the inhibition[(50),(51)]

Procedure:

Table (2-9) : Procedure for determination of urea concentration in serum

	Reagent blank	Standard	sample
Standard	_	10µL	_
sample	_	_	10µL
Working solution	1ml	1ml	1ml

Mixtured and then incubated for 5minutes at (20_25 ^{o}C)

Reagent(NaOH),(NaCLO)	200µL	200µL	200µL

Mixtured and then incubate for 5min at (20_25 ^{o}C) and measurment at wave length 580nm.

Calculation:

$$\text{sample concentration} = \frac{\text{A sample}}{\text{A standard}} \times \text{concentration of standard}$$

2.2.8. Determination of glucose concentration :

Principle: Glucose oxidase catalyses the oxidation of glucose to gluconic acid . the formed hydrogen peroxide (H_2O_2), is detected by a chromogenic oxygen accepter, phenol_aminophenazone in the precence of peroxidase.

$$\beta\text{-D-glucose} + O_2 + H_2O \xrightarrow[\text{catalase}]{\text{glucose oxidase}} \text{gluconice acid} + H_2O_2$$

$$H_2O_2 + \text{phenol} + \text{aminophenazone} \xrightarrow[\text{precence of peroxidase}]{} \text{quinone} + H_2O$$

The intensity of the color formed is proportional to the glugose concentration in the sample[(52),(53).]

Procedure:

(1) Adjust the instrument to zero with distilled water.

(2) Pipette into acuvette:

Table (2-10) : Procedure for determination of glucose concentration in serum

	blank	standard	sample
Working reagent	1.0(ml)	1.0(ml)	1.0(ml)
Standard(µL)	_	1.0	_
sample(µL)	_	_	10

(3)Mixtured and incubated for (20) min at room temperture in (25 ^{o}C).
(4)Readed the absorbance(A) of the samples and standared,against the blank. The color is stable for at least 30 minutes and measurement at wave length is 505nm.

Calculation of concentration of glucose :

$$= \frac{\text{A sample}}{\text{A standard}} \times \text{concentration of standard}$$

2.3.9. Determination of Total protein concentration:

Principle:

Protein gives an intensive violet _blue complex with copper salts in an alkaline medium. Iodide is included as anti oxidant. The intensity of the color formed is proportional to the total protein concentration in the sample[(54),(55).]

Procedure:

(1)Adjust the instrument to zero with distilled water.
(2)Pipette into acuvette:
Table (2-11) : Procedure for determination of total protein concentration in serum

	blank	standard	sample
Reagent(ml)	1.0	1.0	1.0
Standard(µL)	_	25	_
sample(µL)	_	_	25

Reagent(Biuret): sodium potassium tartrate,sodium iodide, potassium iodide, copper(Π)sulphate
(3)Mixtured and incubated 5min or 10 min at room temperture.

(4) Readed the absorbance(A) of the samples and standared,against the blank .The color is stable for at least 30minutes and measurement at wave length is 540nm.

Calculation of concentration of total protien=

$$\frac{\text{(A) sample}}{\text{(A)standard}} \times \text{concentration of standard}$$

2.2.10. Qualitative Analysis of stone:

For a complete chemical analysis,small portion of powdered calculi were added into 5 test tubes to check for the individuals constituents according to the following approaches:

2.2.10.1. Calcium:

1-five drops of hydrocloride acid (10%) were added to the first test tube.
2-Two drops of sodium hydroxide(20%) were added to the same test tube . the appearance of white cloudy precipitate was interpreted as postive for the presence of calcium. [56]

Reagent:

1-10%HCL:preparted by dilution 10ml of concentrated HCL to 100ml with distilled water.

(2)-20% NaOH : prepared by dissolving 20gm of NaOH in 100 ml of distilled water.

2.2.10.2. oxalate and carbonate:

1-To a seconed test tube few drops of hydrochlorid acid (10%) was added.

The appearance of tiny bubbles was interpreted a postive for carbonate.

2-To the residue that remained after heating and cooling , afew drops of hydrochloride acid(10%) were added. The effervescence at this point when there was none before heating were showed the presence of oxalate in stone. **(57)**

2.2.10.3. Uric acid :

1-To a small amount of the pulverized stone ,1 to 2 drops of concentrated nitric acid was added.

2-The solution was evaporated slowly just to dryness, apink_orange color was formed in the presence of uric acid .

3-The tube was cooled and several drops of concentrated sodium hydroxide were poured, apurple color was developed that indicate the presence of uric acid. **(58)**

Reagent :

1-2.5 M NaOH : was prepared by dilution of 156.3ml of 4 N NaOH to 250 ml of distilled water.

2.2.10.4. phosphate:

1-To the fifth test tube 3 drops of 3.5 % ammonium molybdate in 25% nitric acid was added.

2-The tube was heated,the appearance of distinct yellow color was interpreted as positive for phosphate.**(59)**

Reagent:

1-Ammonium molybdate (3.5%):3.5 g of ammonium molybate was dissolved in a solution preparted by mixing 25 ml of concentrated nitric acid with 75ml distilled water .

2.2.10.5 Ammonium ion :

A mmonium ion was detected by the following methode :

1-A small amount of the powdered stone was heated with 2ml of 0.6 M hydrochloric acid.

2-The tube was cooled and neutralized with 2.5M sodium hydroxide .

3-Then 0.5 ml of Nessler solution was added.

4-The apperance of orang –brown precipitate indicated the presence of ammonium in the sample.[58]

Reagents:

1-0.6M HCL :prepared by dilution of 4.95 ml of concentrated HCL to 100ml of distilled water .

2- 2.5M NaOH : prepared as mentioned in section (2.2.10.3)

3- Nessler' s solution prepared by dissolving 100g of mercuric iodiede(HgI_2)

and 70g of potassium iodied (KI) in 400ml of distilled water . The mixture was rotated until a complete dissolving ; 100g of NaOH was dissolved in about 500ml of distilled water.The latter was cooled thoroughly and added with constant shaking to the first mixture. The solution was made up to one liter with distilled water.

2.2.10.6. Determination of calcuim Concentration in urine (24 hours collection):

As described in item (**2.2.3)**.[45]

2.2.10.7. Determination of oxalate concentration in urine(24 hours collection):

Determination of Oxalate concentration was estimated to the permanganate titration method .[60]

1- Five ml of urine sample was mixtured with 5ml of 1 N H_2SO_4.

2- The solution was warmed in (65_80 ^oC),to facilitate the dissolving of oxalate in urine sample.

3- The solution warmed titrated with (0.1 N) $KMnO_4$.

4- The volume of permanganate equivalent volume the sample oxalate in urine, until the pink color of permanganate being stable .

Reagent:

1- potassium permanganate (0.1 N): 0.07 g of potassium permanganate was dissolved in 25ml of distilled water .It was filtered before the use and stored in a dark bottle **.**

2- sulfuric acid (1N): prepared by dilution 14.2ml of concentrated sulfuric acid to 500ml with distilled water.

Calculation:

The concentration of oxalate found in the urine was calculated from the volume of the equivaient potassium permanganate solution.

2.2.9.8 Determination of Total protein in 24 hour urine collection:

Urine Total protein was estimated using precipitation with tri chloro acetic acid(TCA) by Turbid metric method.[61]

Method:

1- One ml of urine sample was taken.

2-Three ml of distilled water was Add and mixture.3-one ml of mixture was pipette and then (4)ml of trichloroacitic acid solution 10% was add ,then the turbidimetry method was read at wave length 450 nm**.**

2.3. Statistical Analysis:

The Result expressed as Means±SD and analyzed statistically. All the biochemical constituent between smoker, sex diffrences and 24 hour urine are analyzed using student's t test. While the influence of age of patient's were expressed by liner regression analysis. Result's less than $p<0.05$ is considered significant.

Chapter Three

Results

3.1. The description of mean percentage of kidney stone composition :

In the current study , 24 urinary calculi were obtained from patients afflicted with Urolithiasis. These calculi were examined for heir shapes. Some shapes of these calculi in which mulberry stone, a jack stone and staghorn stone, which were examined. Composition and constituents of renal calculi were measured by quality analyzed of urinary calculi.

3.2. distribution of individual components in kidney stone:

The frequency of individual component in the quality analyzed kidney stone was expressed in histograms fig(3.1). The percentage of constituent of calcium and oxalate a higher values compared in males with those of females. Uric acid percentages were found to be elevated in males compared with females patients. Phosphate and ammonium showed a higher percentage of these constituent in children compared with patients.

3.3.The effect of sex on the constituent serum of patients with kidney stone:

Kidney stone isolated from males and females were divided according to the sex patients. There were (16) urinary calculi isolated from males and (8) were isolated from females.

The results were evaluated by using student's t test analysis.The analysis of blood sample of patient with renal calculi was pointed out .The highest persentage of constituent of renal calculi is found in male patients compared with female patients .

To study the variations between male and female patients. blood was analyzed by using student's t test analysis.The result of current study pointed out that decreased significantly (P>0.05), in blood glucose in male compared to female patients , Others constituents were appeared to increased level (P<0.05)of all studied parameters in male patients, compared to the female patients. Table(3.1).

3.4. The effect of age on the constituent serum of with kidney stone:

To find the effect of age on the parameter of urinary calculi composition, Patients were categorized into three groups. Group (1) consisted of patients with age 7.0 and up to 19 years, Group (2) contained those with age 20.0 and up to 48 years. Group(3)comprised patients with age 50.0 and up to 60.0 years. To find the relationship between age and constituent of blood of patients with renal calculi, by using liner regression analysis.The linear regression analysis stated a significant positive correlation for glucose ,urea ,uric acid , Creatinine, calcium and total protein with age of patients (r=0.4 ,p<0.05) ,(r=0.4,p<0.05), (r=0.4, p<0.05) (r=0.4, p<0.05), (r=0.06, p>0.05), (r=0.3, p<0.05) while found significant negative correlation for phosphorous, ,acid-phosphate, and alkalin-phosphatate with age patients (r=-0.5, p<0.05), (r=-0.3,p>0.05),(r=0.3,p>0.05), respectively fig (3.2) from (A_I) .

3.5 The effect of smoking on the constituent of serum of patient with_kidney stone:

Un expected result may be related to small number of smoker group of patients with urinary stone .Unfortunately, therefore

, further investigation we need to reveal the above relation.
Table(3.2).

The result of 24 hours and urine collection of (21) patient and (14) individuals as control group .Two groups were matched to age and sex . The analysis was carried out by using student t' test and liner regression Analysis.

The current study exhibited that significant ($P<0.01$) differences between urine calcium of patients compared to the control group. While total protein appeared significant ($P<0.05$).
differences between urine total protien of patients compared to the control group.

.

Additinally urine oxalate concentration analysis was revaled significant ($P<0.05$) of patients when compared to the control group. As shown table (3.3).On the other hand .The differences between patients and control group for the calcium, oxalate and total protien concentration were descripted and through the diagram as shown figure (3.3.a.),(3.3.b.) (3.3.c.) respectively.

Table (3.1) :Shown The effect of sex on the constituent of serum of patient with kidney stone under our study:

parameter	subject	Mean±SD	Range	P- value
Glucose mg/L	Male 20	98.05*±31.599	35 - 170	<0.05
	Female 8	119.38±27.749	95 - 163	
Urea mg/L	Male 20	35.880**±20.6248	15 - 105	N.S
	Female 8	35.000±25.315	18 - 96.0	
Creatinine mg/L	Male 20	1.1200**±0.95955	0.5 -4.5	N.S
	Female 8	0.8500±0.50927	0.4 -2.0	
Uric acid mg/L	Male 20	5.1900**±1.61470	2.4 -7.9	N.S
	Female 8	4.8875±2.11420	3.0 -7.9	
Calcium mg/L	Male 20	8.8750**±1.43486	6.9 -11.5	N.S
	Female 8	8.4875±1.24721	7 - 10.0	

Phosphorous mg/L	**Male 20** **Female 8**	**3.0964**±0.39203** **2.9143±1.04233**	**1.5 -3.2** **1.5 -5.5**	**N.S**
Acid-phosphate U/L	**Male 20** **Female 8**	**4.1090**±1.69615** **3.4175±2.17706**	**1.5 -7.0** **1.04 -7.5**	**N.S**
Alka-phosphate U/L	**Male 20** **Female 8**	**62.7071**±32.582** **59.1464±12.94405**	**15 -124** **30 -113**	**N.S**
Total protien mg/L	**Male 20** **Female 8**	**67.2500**±7.28501** **65.2500±7.54548**	**59 -76** **50 -75**	**N.S**

*P<0.05

**N.S=Non significant

Table (3.2): Shown The effect of smoking on the constituent of serum of patient with kidney stone under our study:

Parameter	Subject	Mean±SD	P- value
Glucose mg/L	**Smoking 4** **Nonsmoking 24**	**110.50±24.960** **103.08±32.865**	**N.S**
Urea mg / L	**Smoking 4** **Nonsmoking 24**	**26.2500±5.67891** **37..1917±22.90384**	**N.S**
Creatinine mg/L	**Smoking 4** **Nonsmoking 24**	**0.7250±0.15000** **1.0958±0.90817**	**N.S**
Uric acid mg/L	**Smoking 4**	**5.3750±1.00125**	**N.S**

	Nonsmoking	**24**	**5.0583±1.84153**	
Calcium mg/L	**Smoking**	**4**	**8.8500±1.11206**	**N.S**
	Nonsmoking	**24**	**8.7500±1.43133**	
Phosphorous mg/L	**Smoking**	**4**	**2.3500±0.55076**	**N.S**
	Nonsmoking	**24**	**3.0083±1.8223**	
Acid- phosphate U/L	**Smoking**	**4**	**3.3875±1.63930**	**N.S**
	Nonsmoking	**24**	**3.9988±1.87927**	
Alk-phosphate U/L	**Smoking**	**4**	**93.0000±34.81379**	**N.S**
	Nonsmoking	**24**	**57.6583±3003223**	
Total protein mg/L	**Smoking**	**4**	**68.2500±6.23832**	**N.S**
	Nonsmoking	**24**	**65.4167±7.60959**	

N.S= Non significant

Table (3-3): Shown The result of 24 hour Mean urine collection for calcium, oxalate, and total protien in patient and control group under our study.

Parameter	Mean ± SD	P-value
Calcium (mg /day)	(patient) 701.719± 627.91 (control) 217.714±38.980	P< 0.01

Oxalate (mg ⁄day)	(Patient) (control)	0.333±0.022 0.213±0.00338	P< 0.05
Total protein(mg⁄day)	(Patient) (Control)	0.7119±0.819 0.322±0.02171	P < 0.05

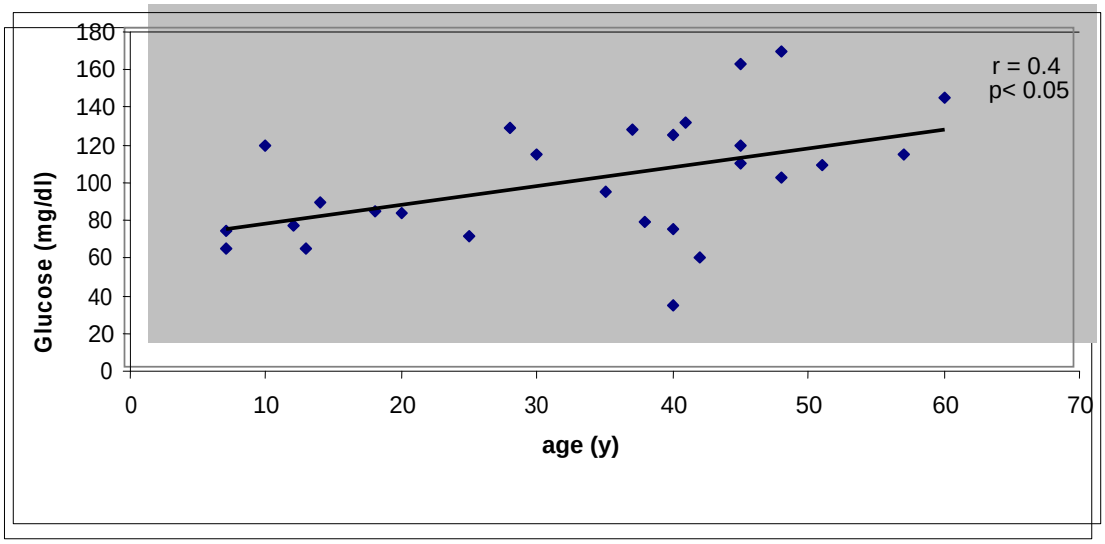


Fig (3.2.A)The relationship between age and glucose concentration.

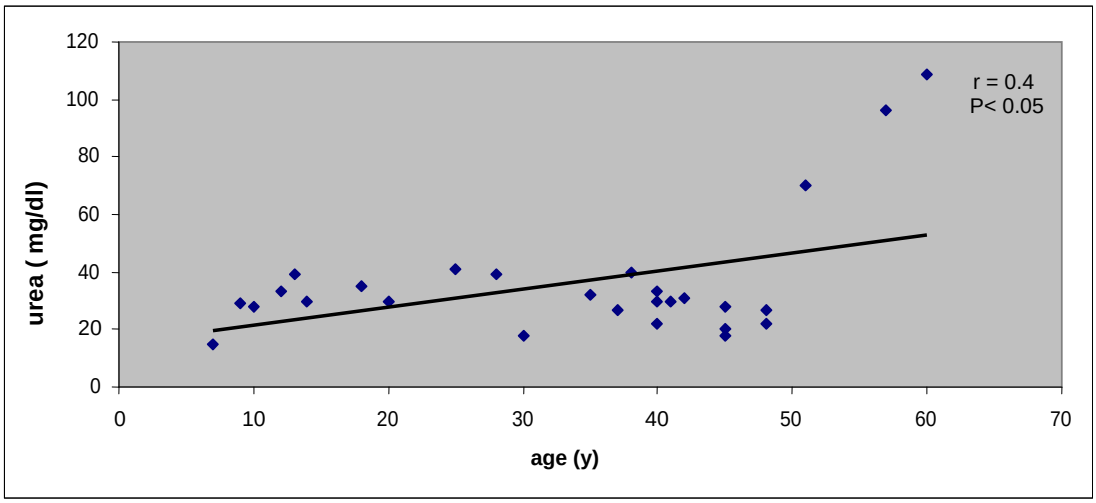


Fig (3.2.B) The relationship between age and urea concentration.

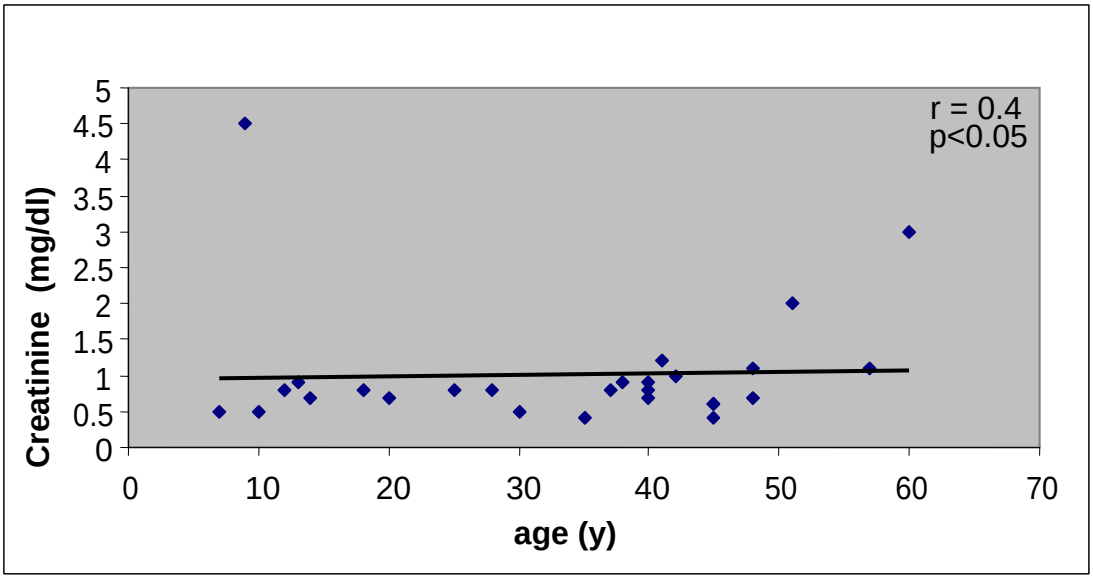


Fig (3.2.C) The relationship between age and Creatinine concentration.

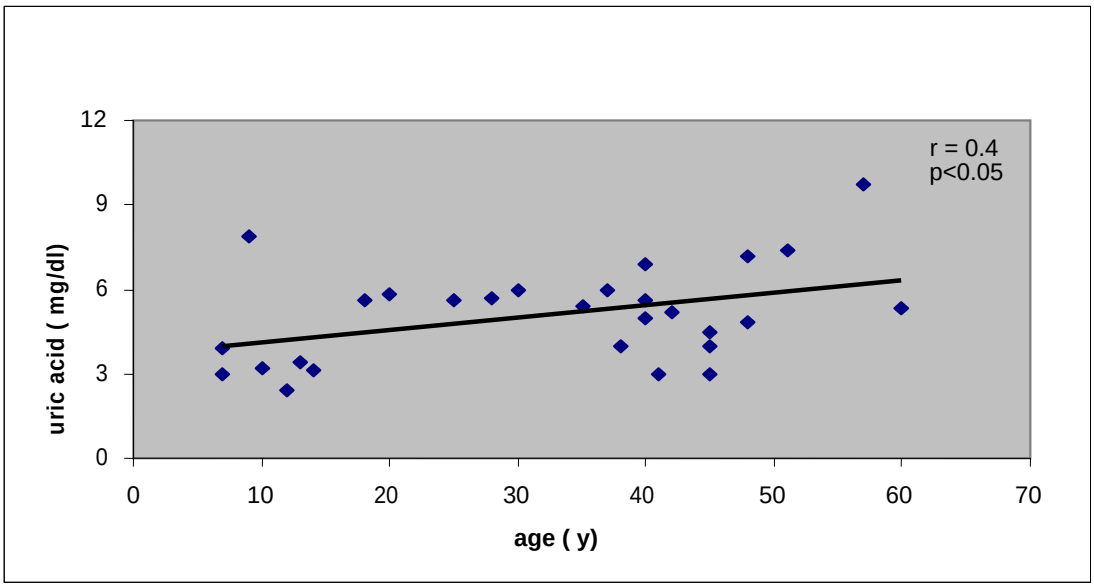


Fig (3.2.D) The relationship between age and uric acid concentration.

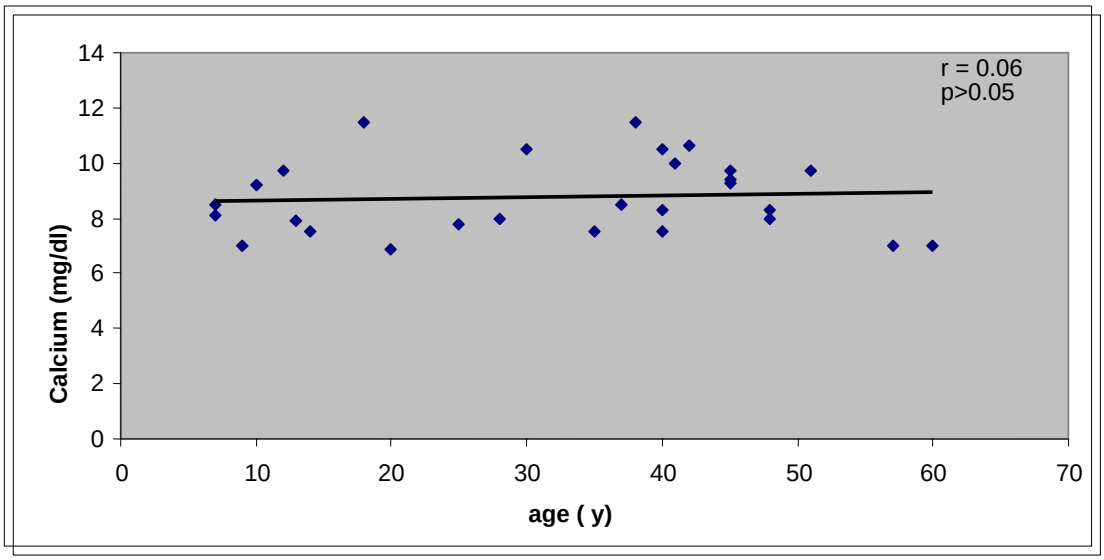
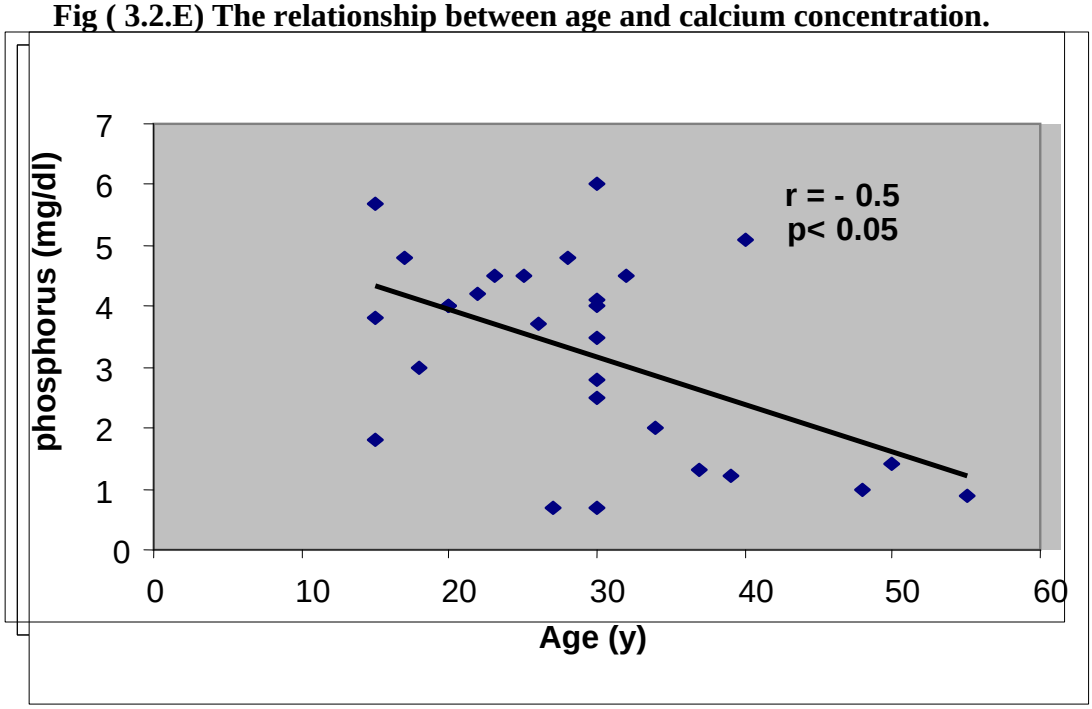


Fig (3.2.E) The relationship between age and calcium concentration.



Fig(3.2.F)The relationship between age and phosphorus concentration.

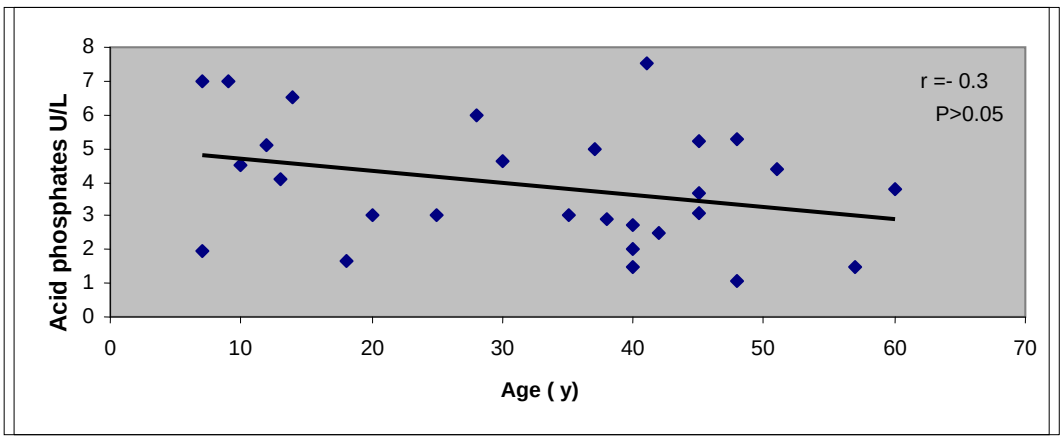


Fig (3.2. G) The relationship between age and Acid phosphates concentration.

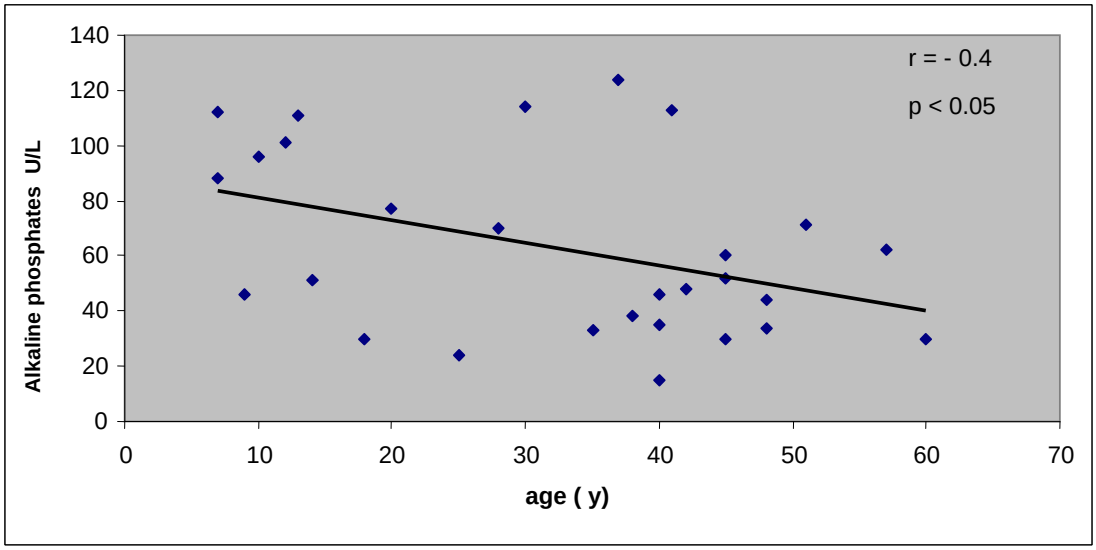


Fig (3.2 .H)) The relationship between age and Alkaline phosphates concentration.

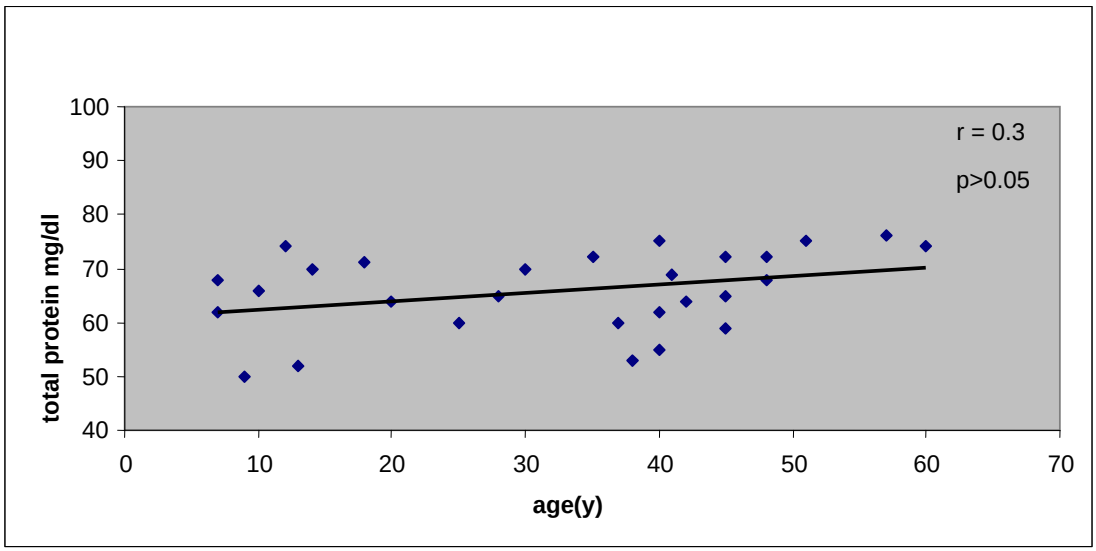


Fig (3.2. I) The relationship between age and Total protien concentration.

Fig[(3. 2) from **(A-I)]**
The relation ship between age and constituent of blood patient with renal calculi.(linear regression analysis).

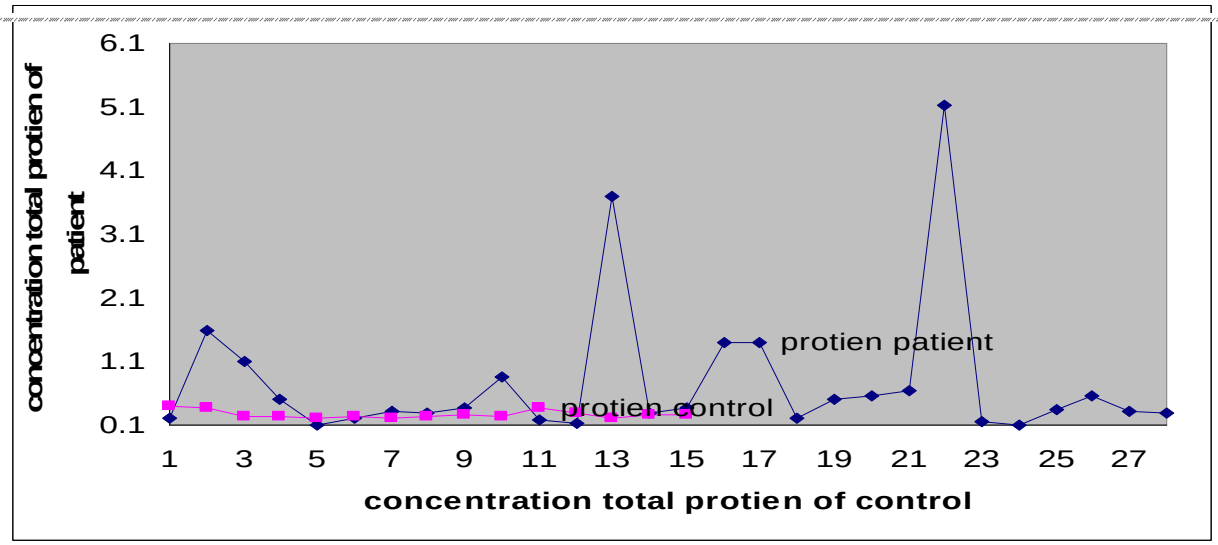


Fig (3.3 a)

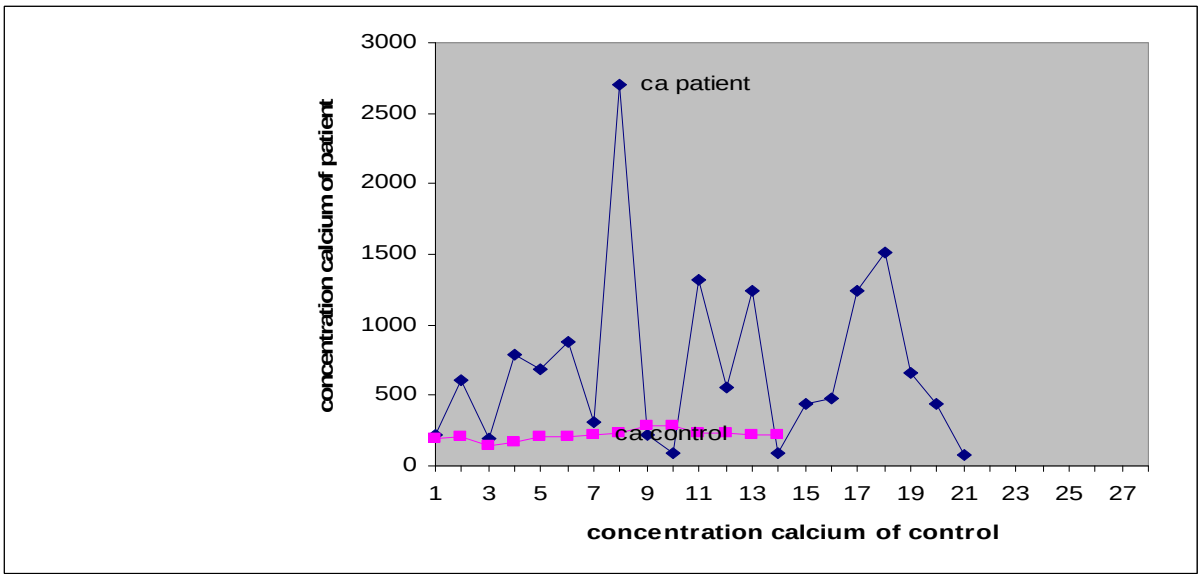
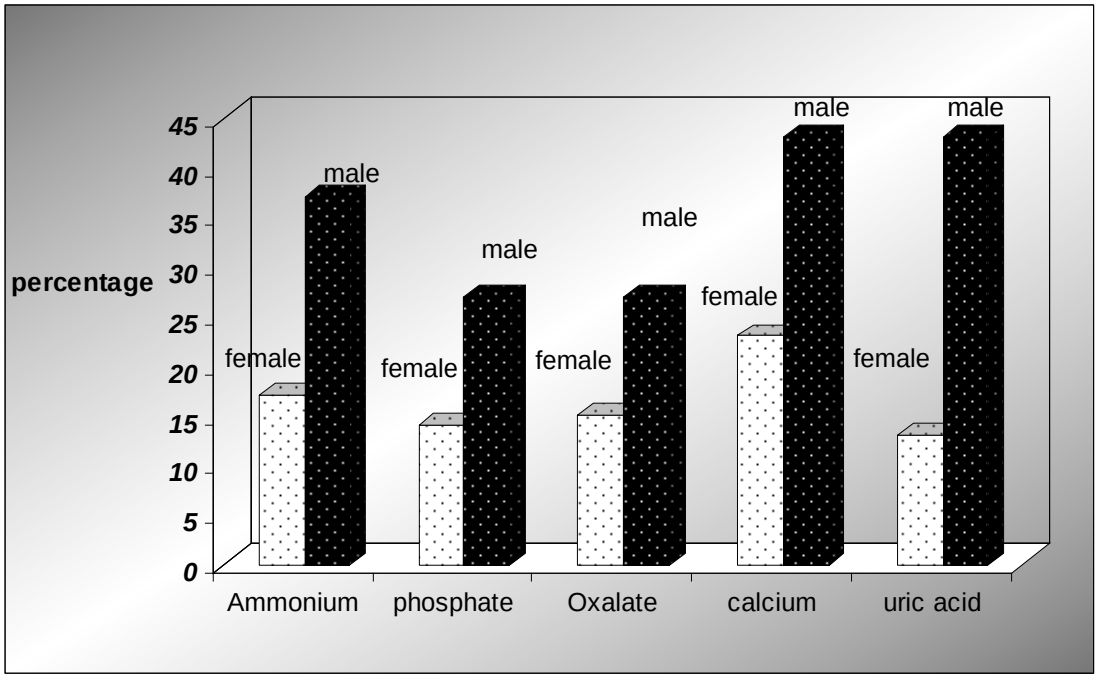


Fig (3.3.b)

Fig (3.3 a,b,c) the relation ship difference between patient concentration and control group a–total protein, b- calcium , c- oxalate (all detail are explained in the text)

Chapter Four

Discussion


percentage
45
40
35
30
25
20
15
10
5
0
female
male
female
male
female
male
female
male
female
male
Ammonium
phosphate
Oxalate
calcium
uric acid

4.1. The description of mean percentage of kidney stone composition:

Kidney stone formation is a complex process, Including crystal Nucleation growth, and aggregation and crystal retention within the renal tubules. [62,63] Crystals form in the urine that supersaturated with particular salts such as calcium oxalate ,calcium phosphate, and urat .[64] calcium oxalate is the most common crystalline components of calculi. In a majority of stone former, the initial step of calculi formation is crystallization of calcium oxalate supersaturated is directly related to the urinary concentrations of calcium and oxalate and inversely related to those of magnesium and citrate. [65] The result of the current study indicated a higher average of calcium and oxalate in male when compared with female patients. However these findings agreement with Hodgkin son and et al [66] had stated a higher average of calcium oxalate from male compared with female patients.

Although calcium oxalate is the major component of most renal calculi,29%of all calculi contain calcium phosphate mostly in the form of hydroxy apatite. [67] It has been proposed that hydroxy apatite forms the nidus of all calcium based calculi, serving as site for heterogeneous nucleation. [68]

Some evidence indicate that an apatite nucleus is present in central nidus of 97% of kidney stone. [69] The other mechanism by which bacterial infection can induce calculi formation is by an alteration in the excretion of the enzymes. Dug Toil et al.[70] had suggested that a factor in Urolithiasis is an

alteration in the excretion of the enzymes urokinase and sialidase.

According to their theory, decreased urokinase and increased sialidase in urine leads to the formation of mineralizable matrix. Bacterial infection with proteous mirabilis and E.coli decrease urokinase and increase sialidase activity**.**

Up to one third of patients with calcium calculi have a history of urinary tract infection, usually associated with E.coli .**(71)** Another explanation is that the deficiency of intestinal bacteria naturally digest oxalate may be the cause of the elevated prevalence of calcium oxalate calculi . When the bacteria are lost due to antibiotic use, the patients is likely to have increased oxalate absorption. **(72)** Other factors may aggravate this phenomenon like the supplement of calcium in women, the sex and the seasonal variation .**(73)** The urinary system is structured in a way that helps ward off infection. The ureters and bladder normally prevent urine from blacking up toward the kidneys, the flow of urine from bladder helps wash bacteria out the body. In men, the prostate gland produces secretions that slow bacterial growth.

Women are found to have a greater occurrence of urinary tract infections Primarily. because the urethra is short ,making it easier for bacteria to reach the bladder **.(74,75)** The results indicated increased phosphate, and ammonium average in children patient. However it can be concluded that the abnormalities in children such as vesicoureteral reflux, urinary obstruction, and present

hematuria that made them with high probability to form infections calculi. [76]

The current investigation demonstrated a high average of uric acid in male than the female patients. The variation was seemed to be age dependent phenomenon. Unforunately calculi protein was not measured in this study.

Several reports demonstrated increased protein average in females compared with males. [77]

However Some authors pointed out that elevated protein content in calculi of female patients may belong to the E.coli infection., this infection may cause glycosaminoglycan layer damage, thereby facilitating bacterial adherence , tissue inflammation, production of organic matrix, and crystal matrix interaction . [78] In some reports, It has been suggested that cystine stone occur only in the patients with cystinuria, unforunately cystinuria was not measured in this study.

The heterozygote for cystinuria generally dose not excrete enough cystine into urine to be increased risk for cystine stone. [79] In addition, It has been found that cystinuria accounting for 6% to 8% of children urinary calculi causes .[80, 81]

4.2. Distribution of individual component in kidney stone:

calcium and oxalate percentages were found to be higher than that of phosphate in the examinated calculi. It is believed that the predominant calcium and oxalate percentages in calculi are related to the nutritional factor in population High protien in take of animal origin contributes to hyperuricosuria,Hypercalciuria , and

hypocitraturia. [82,83] In the past it is recommended calcium restriction

However, it has been to avoid hypercalciuria .[84]

found a reduction in urinary oxalate level associated with increased in take of dietary calcium. [85] Elevated dietary oxalate intake may cause raised oxalate excretion and induces calcium calculi oxalate formation . [86, 87]

The restriction of oxalate intake has been shown to reduce the urinary oxalate excretion ,but do not prevent calculi formation . [88,89] Some authors mentioned other causes of oxalate predominancy in calculi other than the nutritional factor. Intestinal disease may contribute to the promotion of calculi formation due to malabsorption . [90,91]

Uric acid percentages in calculi were found to have high values with respect to other components. This finding may belong to the over ingestion of purine-rich foods. [92] Some times, there is an overproduction of uric acid and hyperuricosuria despite the ingestion of low purine diet. Dehydration may be involved as essential cause for the super saturation of urine with respect to uric acid . [93] The pH of urine may be implicated as a directing factor for the super saturation of urine and crystals formation. [94,95]

4.3. Sex differences of kideny stone composition:

Nephrolithiasis is amulti-factorial disease influenced by environmental as well as hormonal and genetic factors .[65] Its frequency has risen with the development of humanity and varies with the country and geographic area .[96,97] Men are at greatest risk of developing kideny stone with incidence and prevalence rates between two and four times that of women .[98,99]

In the current investigation a higher rate of urinary calculi disease is reported in males than in females, about three males are afflicted for every female. These result are expected to be obtained. Females excrete more citrate and have lower incidence of stone formation than males. Therefore Citrate was found to play several important roles in protecting females against urolothiasis. [100]

In addition, the lower risk of urinary calculi formation in female may be due to the decreased urinary saturation with calculi forming salts. [101] Curhan et al . [102] and Asplin et al . [103] demonstrated decreased incidence of urinary calculi formation in females as compared with males. Baker et al . [104] were found that 70% of all stones analyzed were from men. Men were at greater risk producing calcium oxalate stones and uric acid stones .Women were at greater risk of infection stones .

In the present investigation a higher contents of calcium oxalate and uric acid. The percentage was (79% for male and 21% for female).male when compared with those female calculi.
These result are considered with those reported by Hodgkin son and et al. [66] who reported that a higher average of calcium oxalate in male calculi compared with female calculi. The most likely explanations for these result are that testosterone increase endogenous production by liver. While estrogens decrease urinary oxalate excretion .[105] In addition to that some authors mentioned other causes for calcium oxalate predominancy, Ryall et al . [106] found associated high urinary uric acid concentration with reduced

inhibitory activity of glycosaminoglycans since the latter compounds concentration is more higher in women than in men and consider as naturally inhibitor for calcuim oxalate calculi formation.

4.4.The effect of age on kidney stone compositions:

Renal stone formation is associated with various disorders, including renal tubular acidosis, hypercalciuria ,hyperoxaluria, hypocitraturia, hypomagnesuria, and hyperuricosuria. **(62,107)**

These disorders have a varity of causes but ultimately result in abnormal urinary pH and in excretion of calcium ,oxalate, citrate ,magnesium, and uric acid .**(62)** and its result from a verity of metabolic and nutritional factors in genetically susceptible individuals. **(108)** The most frequent age for kidney stone formation was found (20-48) years. These result are compatible with reported by others (Lavan et al . **(109),** colella .**(110)** The result of current study stated that a higher averages of renal calcium and oxalate contents in the group (20-48) years .The significant negative correlation of calcium and oxalate contents with the patient's age which indicate the lower of urinary excretion of calcium and oxalate concentration with the progression age of patient. However, the data of age study on the composition of renal calculi exhibited higher uric acid contents in old age patient (60-65) years and significant positive correlation of uric acid content with the age of patients.

Thus elderly patients are more prone for formation of uric acid stone rather than calcium oxalate stone. It has been mentioned that the peak of urinary calcium excretion is age (35-45)years, While there is a peak age of endogenous oxalate production occurs at age of 30 years. **(111,112)** Ammonium and phosphate contents in kidney stone were found to have higher average in between (7-18) years. The relation

revealed negative correlation with the age of patients .

These result may reflect a higher excretion of citrate and magnesium in children. The consequences are inhibition of calcium oxalate stone formation and a susceptibility to form other types of stone .[113]

4.5.The effect of smoking on kidney stone compositions:

Many report refers to the clear effect of such parameter. Unlikely ,present data dose not found important effect of smoking habit of the studies parameter. Probably, due to Small population of research and limited time study according to the systematic and low of postgraduate study .

Conclusions

1- Males are more afflicted with renal stone than females .
2- Renal calculi increase gradually with the progress of age .
3- The predominant of uric acid ,calcium and oxalate contents in the studied renal calculi .
4- 24 hours of urine collection reflect clear relationship between hypercalciuria, hyperoxaluria and hyperproteinuria with renal calculi .

Recommendations:

(1)-Establishment of idrological stone therapy center in AL-Forat AL-Awsat .

(2)-More effort of laboratory qualitative analysis of kidney stone.

(3)- Using of technique Fourier transform infrared spectroscopy (FTIR) in kidney stone analysis .

(4)-Public Education about stone formation and treatment.

(5)-Control of chronic disease ,for example A family history of kidney stones , hypertension, history of gout, primary hyperparathyroidism, diabetes mellitus and chronic metabolic acidosis.

(6)- More Studies about Relation between Smoker and Renal calculi.

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الخلاصة

الدراسة الحالية صممت لتقدير المكونات الكيميائية الحياتية لمصل مرضى حصاة الكلى . وتضمنت الدراسة إجراء تقدير الكالسيوم ، الاوكزالات ، البروتين الكلى في الإدرار ٢٤ ساعة لاثنان وعشرون مريضا وقد قورنت مع أربعة عشر من الأصحاء كمجموعة سيطرة .

كما سلطت الدراسة الحالية الضوء على تاثيركل من العمر والجنس والتدخين على مكونات مرضى الحصى الكلوي.كما تضمنت المحاور التالية في الدراسة.

تم إجراء التحليل النوعي لثمان وعشرون حصاة مستخرجة من الكلى وكانت لعشرون رجل وثمان امرأة مصابين بالحصى الكلوي وبأعمار تتراوح من (٧-٦٠) سنة . تم تحليل مصل المرضى بطرائق التحليل الكمي، واستخدمت البيانات لحساب كل من الكلوكوز،اليوريا،الكرياتنين،حامض اليورك،الكالسيوم،الفسفور،البروتين الكلى .وكذلك تضمنت الدراسة قياس مستوى الإنزيمات الفوسفات الحامضى،الفوسفات القاعدي. أظهرت النتائج تقدير تركيز الكلوكوز زيادة معنوية ($P<0.05$) في الجنس.

أوضحت دراسة تأثير العمر على المكونات الكيماوية الحياتية وكانت تشير زيادة معنوية($p<0.05$) موجبة الترابط مع العمر لكل من سيرم الكلوكوز، اليوريا، الكرياتنين، حا مض اليوريك، الكالسيوم والبروتين الكلي وبينما أظهرت انخفاض معنوي ($p>0.05$) سالب الترابط مع العمر لكل من سيرم الفسفور ،الفوسفات الحامضى والفوسفات القاعدي،إن تأثير عامل التدخين لم يظهر اى تغيرات على المكونات المدروسة .

كما بينت نتائج تحليل الادرار٢٤ ساعة زيادة معنوية ($P<0.01$)،($P<0.05$) لكل من تركيز الكالسيوم،الاوكزالات،البروتين الكلى لمرضى حصاة الكلى عند مقارنتها مع مجموعة السيطرة.

أظهرت دراسة التحليل النوعي للحصاة المدروسة زيادة نسبة كل من حامض اليورك، الكالسيوم، الاوكزالات، الفسفور، الامونيوم لحصاة الذكور عند مقارنتها مع حصاة الإناث.

أوضحت الدراسة بما يلي:إنشاء مركز لعلاج حصى المجارى البولية في الفرات الأوسط ، التوعية الصحية للسكان ،تحليل كمي لحصى الكلية، استعمال تقنية أشعة تحت الحمراء في تحليل الحصى ،السيطرة على الإمراض المزمنة مثل ارتفاع السكر،التاريخ العائلي،ارتفاع الضغط ، التاريخ للنقرص، ارتفاع افرازالغدة الجنب الدرقية ،الحموضة المزمنة للهضم . دراسات أكثر للعلاقة بين المدخنين وحصاة الكلى.

بسم الله الرحمن الرحيم

ويسألونك عن الروح قل الروح من أمر ربي وما أوتيتم من العلم إلا قليلاً. سورة الإسراء

صدق الله العلي العظيم

وزارة التعليم العالي والبحث العلمي
جامعة كربلاء - كلية العلوم
قسم الكيمياء

دراسة مقدمة

إلى مجلس كلية العلوم - جامعة كربلاء

كجزء من متطلبات نيل درجة الدبلوم العالي في التحليلات الكيميائية والدوائية

من قبل

حيدر ناجي خضير

بكالوريوس علوم الكيمياء / جامعة البصرة

١٩٩١

بأشراف

الأستاذ الدكتور
صاحب علي
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الاستاذ الدكتور
صالح مهدي حداوى

نيسان ١٤٣١هـ

April ٢٠١٠م