

Ministry of Higher Education and Scientific Research
University of Diyala
College of sciences

**Determination of Urae ,Creatinine,
Albumine, Glomerular Filtration rate and
Calcium in the patients with Chronic Renal
Failure**

A Project

Submitted to the Department of Chemistry -College of
sciences/ University of Diyala . In Partial Fulfillment of
the requirements for the degree of B. Sc. in Chemistry

By

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اٰیة ۱۰۹ سورۃ الکہف

كُلًّا نُّنَزِّلُ لِطَآئِفَتِهِمْ رَبِّ السَّمَوٰتِ وَالْاَرْضِ
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صدق الله العظيم

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بِسْمِ اللّٰهِ الرَّحْمٰنِ الرَّحِیْمِ

الإهداء

الى من جرع الكاس فارغاً ليسقيني
الى من كلت انا مله ليقدّم لنا لحظة سعادة
الى من حصد الاشواك عن دربي ليهد لي طريق العلم
الى القلب الكبير..... والدي.

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الى القلب الناصع بالبياض..... والدتي الحبيبة

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حياتي..... اخوتي

الآن تفتح الأشعة وترفع المرساة لتنطلق السفينة في عرض بحر واسع
مظلم هو بحر الحياة وفي هذه الظلمة لا يضيء الاقنديل الذكريات
ذكريات الاخوة البعيدة الى الذين احببتهم واحبوني.....

اصدقائي

الى الأرواح التي سكنت تحت التراب تراب الوطن العظيم.....
الشهداء العظام

اليكم جميعاً أهدي هذا العمل

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Abstract

The aim of study is to determine the concentration of total calcium, urea, creatinine , albumin and glomerular filtration rate (GFR) in the serum of patients suffering from chronic renal failure . 75 specimens of each of suffering and control groups were collected. Patients were divided into two study groups dependent on treatment time: (first) regulated blood washing less than year group and (second) regulated blood washing more than year group. The study showed an increase in creatinine ($120.2 \pm 7 \mu\text{mol/L}$) ,urea($29.75 \pm 1.3 \text{mmol/L}$) concentration in the serum of suffering group when compared with control group creatinine was($60 \pm 05 \mu\text{mol/L}$)and urea ($4.5 \pm 0.13 \text{mmol/L}$). The results also showed a decrease in the concentration of total calcium($3.55 \pm 1.01 \text{mg/dl}$)and glomerular filtration rate($25.15 \pm 13 \text{ml/min}$) compared with control group total calcium was($9.02 \pm 1 \text{mg/dl}$) and glomerular filtration rate($123 \pm 5 \text{ml/min}$). There were a negative correlation between GFR and each of (age, creatinine, and urea) in both patients groups .Also There were a positive correlation between GFR and total calcium in both patients groups

1-Introduction

Renal failure is a condition in which the kidneys fail to remove metabolic end-products from the blood and regulate the fluid, electrolyte, and pH balance of the extracellular fluids. The underlying cause may be renal disease, systemic disease, or urologic defects of nonrenal origin⁽¹⁾. Renal failure can occur as an acute or a chronic disorder. Acute renal failure is abrupt in onset and often is reversible if recognized early and treated appropriately. In contrast, chronic renal failure is the end result of irreparable damage to the kidneys. It develops slowly, usually over the course of a number of years⁽²⁾. Causes may include diseases such as diabetes, high blood pressure and heart disease ,and Kidney stones Blockage or problems in the urinary tract⁽³⁾ .proteins are an important factor because it is a measure of the levels of calcium in the plasma. The calcium metal element is affected by absorbed on many factor, Including the degree of acid ,the concentration of phosphate and vitaminD. Abnormalities of calcium, phosphate, and vitamin D occur early in the course of chronic renal failure. They involve the renal regulation of serum calcium and phosphate levels, activation of vitamin D, and regulation of parathyroid hormone (PTH) levels. The regulation of serum phosphate levels requires a daily urinary excretion of an amount equal to that absorbed from the diet. With deteriorating renal function, phosphate excretion is impaired, and as a result, serum phosphate levels rise. At the same time, serum calcium levels fall because serum calcium is inversely regulated in relation to serum phosphate levels. The drop in serum calcium, in turn, stimulates PTH release, with a resultant increase in calcium resorption from bone. The kidneys regulate vitamin D activity by converting the inactive form of vitamin D [25(OH)

vitamin D3] to its active form (1,25-OH₂vitamin D3). Decreased levels of active vitamin D lead to a decrease in intestinal absorption of calcium with a resultant increase in parathyroid hormone levels. Vitamin D also regulates osteoblast differentiation, thereby affecting bone matrix formation and mineralization.⁽⁴⁾⁽⁵⁾ Measured total calcium in serum consists of 15% bound to organic and inorganic anions, about 40% bound to albumin, and the remaining as biologically active ionized calcium. A variety of formulae have been proposed to permit calculation of the albumin corrected total calcium or ionized calcium from the total calcium and protein concentration, but no data support the use of such algorithms^(6,7)

glomerular filtration rate (GFR) is one of the most important functions of the kidney , where the outputs of crap of the processes of metabolism through the blood, to the glomeruli of the renal tubules happens filtration there and going through the pipes small renal and out of without being re-absorbed . But what happens to urea it's re-absorbed, in part, through pipe renal . Leading to not rely entirely on the examination of the urea in the evaluation of the filtration function resort to the examination of the work, creatinine, which totally filtered without is absorbed again by the tubes of the renal tubules⁽⁷⁾⁽⁸⁾ . Urea is a product of the removal of ammonia from amino acids Which represents the final product to undermine the protein manufacture of urea from ammonia in the liver and excreted through the kidney⁽⁸⁾. Range of urea in the blood normal (14-50 mg/100 ml) can be considered as the upper limit in the limits of the(50 mg/100 ml) when people chief of the age⁽⁹⁾. Increase in the rate of urea in the blood in renal failure acute have been up to(500mg/100 ml)^(10). Creatinine different form urea in the to the quantity in the urin dependent on representation of proteins of the

internal. While urea is dependent on external protein⁽¹¹⁾. Creatinine, a by-product of muscle metabolism, is freely filtered in the glomerulus and is not reabsorbed in the renal tubules. Creatinine is produced at a relatively constant rate, and any creatinine that is filtered in the glomerulus is lost in the urine, rather than being reabsorbed into the blood. Thus, serum creatinine can be used as an indirect method for assessing the GFR and the extent of renal damage that has occurred in renal failure. Because creatinine is a by-product of muscle metabolism, serum values vary with age and muscle mass. An increase in serum creatinine to three times its normal value suggests that there is a 75% loss of renal function, and with creatinine levels of 10 mg/dL or more, it can be assumed that 90% of renal function has been lost⁽¹²⁾⁽¹³⁾

2-Material and Method

2.1 patients and control

This study has been carried out at the consultative clinic in Baquba/Ibn seena center for renal failure , for the period January 2016 to march ,2016. The study included (55) patients (females and males) with age range (35-70) year.20 Iraqi control subjects comparable to patients in respect to age (35-70 year) and gender , were included in the study.

Patients were divided into two study groups dependent on treatment time : (first)regulated blood washing less than years group and (second) regulated blood washing more than years group.

First group/ included 32 subjects aged 40- 69 years (mean age 54.31 second group/included 23 subjects aged 40- 69 years (mean age 54.31 years) from each subject, 5 ml of blood were obtained by venepuncture, using a 10 ml disposable syringe between 9.00 and 11.00 A.M. The blood sample was dispensed in a plain tube and left for around an hour to clot at room temperature (22°C). Then, it was centrifuged at 3000 rpm for 10 minutes to collect serum. The serum was divided into aliquots (50µl) in Eppendorff tubes and use to measure

2.2 Methods and Biochemical Determinations

2.2.1 Measurement of Body Mass Index (BMI)

BMI uses a mathematical formula based on a person's height and weight. BMI equals weight in Kilograms divided by height in square meter (BMI=Kg/m²)^(1 4). [WHO; and Fost] suggested that a BMI of 18.5–24.9 indicates a person of normal weight. A person with a BMI of 25–29.9 is overweight, while a person with a BMI of ≥ 30 is obese^{(15) (16)}

2.2.2 Measurement of serum creatinine concentration

Creatinine forms a colored orange-red complex in alkaline picrate solution. The difference in absorbance at fixed times during conversion is proportional to the concentration of Creatinine in the sample.

Reagents composition

Reagent	Contents	Initial concentration of solutions
CAL	Standard	173 μ mol /L (1.98 mg/dL)
R1a	Picric acid	35 mmol/L
R1b	Sodium hydroxide	0.32 mol/L

Procedure: Let reagent and specimens stand at room temperature

Reagents	Standard	Sample
Working Reagent (WR)	1.0 ml	1.0 ml
Standard Solution	0.1 ml	--
Sample	--	0.1 ml

let stand for 5 minutes at 37C^o or 10 minutes at room temperature. Record absorbance at 49 nm (480-520) against reagent blank.

Calculation:

Calculate the result as follows:

Men : 53-97 μ mol/L Women : 44-80 μ mol/L

2.2.3 Measurement of serum urea concentration

Serum urea was determined utilizing a ready-made laboratory kit for this purpose, the principle of determination was based on the enzymatic hydrolysis according to the following reaction



Reagents composition

Reagent	Contents	Concentration of Solution
CAL Standard	Urea	8.33 mmol/L (0.5 g/L)
R 1a Enzymes	Urease	>350 kU/L
R 1b Color reagent	Phosphate buffer PHS Sodium salicylate Sodium nitroprusside EDTA	50 mmol/L 62 mmol/L 3.35 mmol/L 1 mmol/L
R 2 Alkaline reagent	Sodium hydroxide (NaOH) Sodium hypochlorite (NaClO)	0.5 mol/L 24.8 mmol/L

Procedure: Let reagent and specimens stand at room temperature

	Reagent blank	Standard	Sample
Standard	–	10µl	–
Sample	–	–	10µl
Working Solution	1ml	1ml	1ml
Mix			
Incubate for 3 minutes at 37 c°			
Reagent 2	200 µl	200 µl	200 µl
Mix			
Incubate for 5 minutes at 37c°			
Perform photometry			

The absorbance was read at 600 nm against blank solution the intensity of color developed is proportional to the concentration of urea in the patient sample

Calculation:

Calculate the result as follows:

$$\text{urea Concentration (mmol/L)} = \frac{A \text{ for Specimens}}{A \text{ for standard}} \times 8.33$$

Normal Value 2.5 – 7.4 mmol/L

2.2.4 Measurement of serum albumin concentration

The albumin were determined in serum by using Broom Cresol Green (BCG) method. Reacts BCG with albumin in acid solution. the intensity of color developed is proportional to the concentration of albumin in the patient sample.

Reagents composition

Reagents	Contents	Concentration of Solution
R	bromcresol green ph 4.2	0.12 mmol/L
Albumin CAL	albumin aqueous primary standard	5 g /dL

Procedure: Let reagent and specimens stand at room temperature

Reagents	Blank	Standard	Sample
R(ml)	1.0 ml	1.0 ml	1.0 ml
Standard	---	5 µl	---
Sample	---	---	5 µl

let stand for 10minutes at 25C^o temperature .The absorbance of test was read at 630 nm spectrophotometer

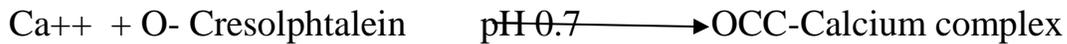
Calculation:

Calculate the result as follows albumin Concentration (g/dl)= A for Specimens/ A for standard ×5

Normal value (3.5 –5) g/dL

2.2.5 Measurement of serum calcium concentration

The method is based on the specific binding of O Cresolphthalein complexone (OCC) and calcium at alkaline PH with resulting shift in the absorption wavelength of complex . The intensity of color developed is proportional to the concentration of total calcium in the patient sample.



Reagents composition

Reagent	Contents	Concentration of Solution
R 1 Buffer	Ethanolamine	500 mmol/L
R 2 Chromogen	O- Cresolphtalein 8- Hidroxyquinolein	0.62 mmol/L 69 mmol/L
Calcium CAL	Calcium aqueous primary standard	10mg/L

Procedure: Let reagent and specimens stand at room temperature

Reagents	Blank	Calibrator	Sample
R 1 (ml)	1.0	1.0	1.0
R 2 (ml)	1.0	1.0	1.0
Calibrator (µL)	---	20	---
Sample(µL)	---	---	20

let stand for 2minutes at room temperature .The absorbance of test was read at 570 nm against the reagent blank.

Calculation:

Calculate the result as follows total calcium Concentration (mg/dl)= A for Specimens/ A for standard ×10

Normal value (8.4 –10.2) mg/dL

2.2.6 Estimate glomerular filtration rate (GFR)

Estimate glomerular filtration rate (GFR) can be calculated mathematically from the Modification of Diet in Renal Disease (MDRD) formula

(GFR (ml/min/1.73 m²) =186×(Serum creatinine)^{-1.154} ×(Age)^{-0.203} ×(0.742 if female)×(1.210 if black).

Normal Value : 100 –130 ml/min⁽¹⁸⁾

3-Result and Discussion

The clinical characteristics and biochemical parameters of kidney function from the whole chronic renal failure group and from the control group are summarized in Table 1. Age, BMI, Total calcium, Albumin, Urea, creatinine and GFR were comparable between the patients group and control subjects. The mean age for patients group was (51.05 ± 16.344) years which was comparable to that of healthy control mean (49 ± 14.1) years. The patients and the control were age matched. It is clear that no significant differences in This means that the research sample homogeneous among themselves. There are no significant differences in BMI when comparing patients with non patients counterparts as shown in table (1). The same table revealed that mean total calcium for patients group was (3.55 ± 1.019027) , while the total calcium for control group was found to be (9.02 ± 1) . The difference was highly statistically significant ($P < 0.001$). Result were in agreement with Van Hooft (1993) who stated that low levels of calcium are found in people with chronic renal failure. The reason for the low calcium to low absorbed in the intestines. Where metabolic of calcium has a strong relationship with metabolic of phosphor, the absorption of calcium and phosphorus is in the intestines and must not exceed the ratio of calcium to phosphorus is about 1:2. Albumin was found to be lower in patients compared with the healthy subjects but this difference no statically significantly as shown in table (1). Fall albumin as a result of low calcium where 50% of the calcium in the blood plasma is connected with the albumin⁽¹⁹⁾. The mean GFR in patients group was found to be significantly elevated when compared with healthy subjects. The results of the present study are similar to observation of other investigators, Guyton (2000) et al. who explained that Renal failure develops when the GFR is less than 20% of

normal. At this point, the kidneys cannot regulate volume and solute composition, and metabolic acidosis. These alterations affect other body systems to cause neurologic, gastrointestinal, and cardiovascular manifestations⁽²⁰⁾. These lower in GFR which in turn lead to increasing in serum creatinine and urea. Serum creatinine and urea were found to be higher in patients group compared with the healthy subjects (P < 0.001) as shown in table (1). The mean creatinine for patients group was (120.2±7) which was comparable to that of healthy control mean (60±05) and the mean urea for patients group was (29.75±1.3) which was comparable to that of healthy control mean (4.5±0.13). The results of the present study regarding serum creatinine and urea are similar to observations of other investigators, Kolagal, et al (2009) who stated that serum urea elevated in CRF⁽²¹⁾. The accumulation of nitrogenous wastes is an early sign of renal failure, usually occurring before other symptoms become evident. Urea is one of the first nitrogenous wastes to accumulate in the blood, and level becomes of urea increasingly elevated as renal failure progresses. The increase in creatinine concentration in serum blood of patient to the fact that creatinine of waste metabolic posed naturally with urine. In the case of renal failure gets a defect in the kidney to prevent her from doing filtration and curiosity to the outside leading to high creatinine in blood serum⁽²²⁾⁽²³⁾

Table 1-Characteristics of study subjects

variable	mean ± SD Patients group	mean ± SD Control group	P value
Age (years)	51.05769±16.34	49±14	NS
BMI(kg/m ²)	21.405± 5.01	24.32± 1.2	NS
Total calcium(mg/dl)	3.55±1.01	9.02±1	P<0.001
Albumin(g/dl)	3.44±2.77	4.1±02	NS
Urea(mmol/L)	29.75±1.3	4.5±0.13	P<0.001
Creatinine(μmol/L)	120.2±7	60±05	P<0.001
GFR(ml/min)	25.15±13	123±5	P<0.001

The clinical characteristics of kidney function from the first group (regulated blood washing less than years), second group (regulated blood washing more than years) and from the control group are summarized in table(2) . Age, BMI, Total calcium, Albumin ,Urea ,creatinine and GFR were comparable between the three group.

As shown in table 2, no significant variations were observed between the means of age in the two groups of patients and controls. Also no significant variations were observed between the means of albumin in the two groups of patients and controls. The serum calcium showed a gradual decreased level in two groups of CRF patients (4.4 ± 0.12 and 3.63 ± 0.48 , respectively) compared with controls(9.02 ± 1). Significant difference($P < 0.05$) was recorded (Table 2). Result were in agreement with Van Hooft (1993)⁽¹⁹⁾ . BMI was found to be normal in CRF patients compared with the healthy subjects and significantly lower in second group patients than first group patients and healthy subjects ($P < 0.05$) as shown in table 3. This lower may be to inflammation which is one of important causes to hypoalbuminemia and loss of appetite in patients with washing blood more than years⁽²⁴⁾ . serum creatinine ,urea ,and GFR were found to be higher in patients compared with the healthy subjects and significantly elevated in second group patients than first group patients and healthy subjects ($P < 0.05$) as shown in table 2. As shown in table 2, these result may be to when the kidneys lose most of their function, called end-stage renal failure, dialysis is needed several days a week. A kidney transplant may also be a treatment option⁽²⁵⁾ .

Table(2)Clinical characteristics of patients with CRF In the two group and control group

Variable	First group	Second group	Control group	P value
Age(years)	51.44±16.44962	53±8	49±14	NS
BMI(kg/m ²)	24±3	19±2	24.32± 1.2	P<0.05
Total calcium(mg/dl)	4.4±0.12	3.63± 0.48	9.02±1	P<0.05
Albumin(g/dl)	5.764±1.8	4.949±0.9	4.1±	NS
Urea(mmol/L)	31±37	31.4±39	4.5±0.13	P<0.05
Creatinine(μmol/L)	110±2	120±5	60±05	P<0.05
GFR(ml/min)	33±4	16±5	123±5	P<0.05

*Significant using ANOVA test at 0.05 level of significance

In figure (1) we notice that there was a highly significant difference (P<0.05) between patients in BMI ranges, most of patients in first group were in normal range (50%). And 37% of them were in over weight. Few of patients were in range less than 18. Our results are agree with the Kaur ⁽²⁴⁾

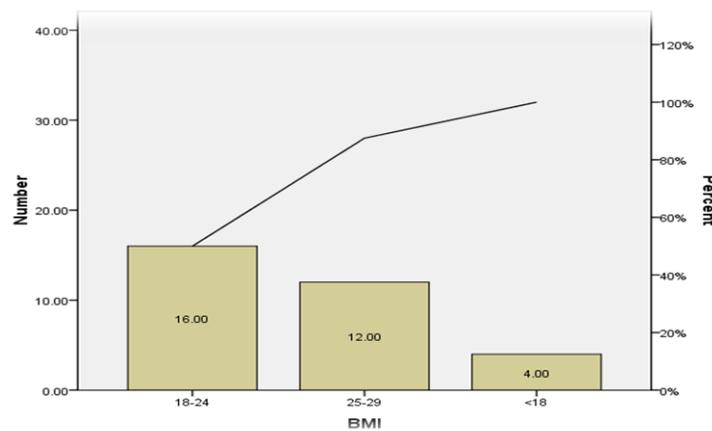


Figure 1-Distribution of patients according BMI (Kg/m²) in first group (Comparison of significant by Kruskal-Wallis Test

In figure (2) we notice that there was a highly significant difference ($P < 0.05$) between patients in BMI ranges, most of patients in second group were in normal range (47%). And 6.8% of them were in over weight. 43.4% of patients were in range less than 18. Our results are agree with the Lesley (2012) ^(24 a) most of patients were in normal range or lower of normal range this may be to the larger proportions of the patients were older and had diabetes,

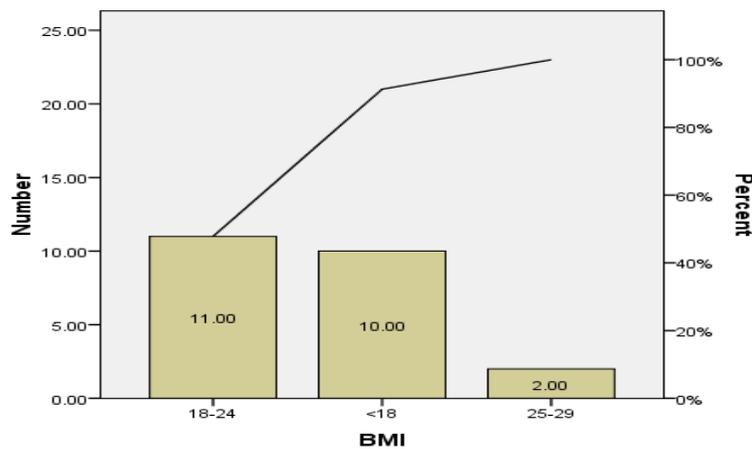


Figure 2 Distribution of patients according BMI (Kg/m^2) in second group (Comparison of significant by Kruskal-Wallis Test)

The statistical analysis showed significant correlation between GFR and age in first group and second group ($r=0.656, p=0.00$ and $r=0.425, p=0.022$ respectively), as shown in table 4. Among older persons, the presentation and course of renal failure may be altered because of age-related changes in the kidneys and concurrent medical conditions. Normal aging is associated with a decline in the GFR and subsequently with reduced homeostatic regulation under stressful conditions. This reduction in GFR makes elderly persons more susceptible to the detrimental effects of nephrotoxic drugs, such as radiographic contrast compounds. The reduction in GFR related to aging is not accompanied by a parallel increase in the serum creatinine level

because the serum creatinine level, which results from muscle metabolism, is significantly reduced in elderly persons because of diminished muscle mass and other age-related changes. Evaluation of renal function in elderly persons should include a measurement of creatinine clearance along with the serum creatinine level^(25a). Also there was significant negative correlation ($r = -0.881$, $p = 0.002$) between GFR and serum creatinine, while there was a significant positive correlation

between GFR and Total calcium in first group ($r = 0.781$, $p = 0.000$) as shown in table 4. On the other hand, table 4 showed no significant correlation between serum albumin and GFR ($r = 0.132$, $P = 0.530$ and $r = 0.190$, $p = 0.386$, respectively) in first and second group. A significant positive correlation in second group has been shown between total calcium and GFR ($r = 0.821$, $p = 0.00$). On the other hand, there was significant negative correlation between GFR and each of the creatinine and urea parameters ($r = -0.704$, $p = 0.002$ and $r = -0.523$, $p = 0.04$ respectively) as shown in table 4. Our results agree with the Stevens et al (2005)⁽²⁶⁾. Chronic renal failure represents the end result of conditions that greatly reduce renal function by destroying renal nephrons and producing a marked decrease in the glomerular filtration rate (GFR). The accumulation of nitrogenous wastes is an early sign of renal failure, usually occurring before other symptoms become evident. Urea is one of the first nitrogenous wastes to accumulate in the blood, and the level of urea becomes increasingly elevated as renal failure progresses. Creatinine, a by-product of muscle metabolism, is freely filtered in the glomerulus and is not reabsorbed in the renal tubules. Many studies support the similarity of creatinine clearance to GFR and its reciprocal relationship with the serum creatinine level. Creatinine is secreted by proximal tubular cells as well as filtered by the glomerulus; thus, the

creatinine clearance exceeds the GFR. Tubular secretion of creatinine varies among and within individual persons, especially in those with a mild-to-moderate reduction in the GFR⁽²⁷⁾. Abnormalities of calcium occur early in the course of chronic renal failure. When GFR decreased serum phosphate levels rise. At the same time, serum calcium levels fall because serum calcium is inversely regulated in relation to serum phosphate levels. The drop in serum calcium, in turn, stimulates PTH release, with a resultant increase in calcium resorption from bone⁽²³⁾

Table 4 showed correlation between GFR and variable (age ,Albumin,creatinine,urea and Ca) in two group of patients with CRF

Variable	First group GFR		Second group GFR	
	Pearson Correlation		Pearson Correlation	
Serum Albumin	r	0.132	r	0.190
	p	0.530	p	.386
Serum urea	r	-.105	r	-.55*
	p	0.624	p	.04
Age	r	0.656**	r	.425*
	p	0.000	p	.022
Serum creatinine	r	-.881**	r	-.704**
	p	0.002	p	.002
Ca	r	.0.781**	r	.0.821**
	p	0.000	p	0.000

** . Correlation is significant at the 0.01 level (2-tailed).

* . Correlation is significant at the 0.05 level (2-tailed).

Conclusions

Through what has been previously reported study found asset of conclusions can be summarized as follows :-

1-This study concludes high concentration of both urea and creatinine in patients with chronic renal failure .

2-Also it noted a decrease in the concentration of calcium and glomerular filtration rate and albumin. .

RECOMMENDATIONS

The study concluded a number of recommendations that carry scientific insights and ideas can be summarized palate :-

1-Conduct a study on biological variables calcium and albumin ,urea and total protein among dialysis patients before dialysis session and then.

2-Healthy food and maintain the heart and blood vessels and prevent diabetes which hurt the performance of the kidney .

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الجنس

اسم المريض

الطول

الوزن

العمر

حاد

فترة الاصابة بالمرض

مزمن

كم مرة تم فيها غسل الكلية

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نوع العلاج

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هل مصاب بامراض اخرى غير الفشل الكلوي

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يوريك اسيد

كيراتينين

البوريا

