

# Evaluation of Serum Procalcitonin and Other Kidney Function Test in Patients with Large Renal Stone

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## ABSTRACT

**Background:** Renal stone disease is a main cause for acute kidney injury. Renal stone can damage the tubular epithelial cells, which can lead to functional loss of the renal parenchyma. Procalcitonin (PCT) is a peptide precursor of the hormone calcitonin. PCT levels are marker for determine if a patient has an infection, bacteria selectively aggregate to crystal and that bacteria are associated with an increased number of crystal-crystal agglomerations. Bacteria-crystal aggregates bind to the tubular epithelium resulting in expression of stone matrix proteins in either renal tubular epithelium or inflammatory cells.

**Objective:** To estimate serum procalcitonin (PCT) in primary renal stone patients and compare it with secondary renal stone group when renal stone size  $\geq 1.5$  cm.

**Subjects, Materials and Methods:** This Cross-sectional Study was designed incorporation with college of Medicine, Mustansiriya university, chemistry and biochemistry Department. Samples were taken from Al Yarmouk teaching hospital in Bagdad, urology department. A total number of eighty eight (88) patients were registered (44 male) and (44 female) aged between (30-40) years. The total subjects were divided into two groups: Group I: Patients with Primary Renal Stone, Group II: Patients with Recurrent(secondary) renal stone.

**Results:** Analysis of variance for serum PCT were significant,  $p \leq 0.0001$ , indicating there were significant difference in serum PCT among renal stone size in primary renal stone group and recurrent(secondary) renal stone group.

**Conclusion:** Serum procalcitonin increased significantly in patients with recurrent renal stone disease, therefore, it is considered as a predictor of kidney stone disease.

**Keywords:** Serum Procalcitonin, Primary Renal stone, Secondary Renal stone, Serum uric acid, and Serum Albumin.

## INTRODUCTION

Procalcitonin (PCT) is a peptide precursor of the hormone calcitonin, the latter being involved with calcium homeostasis. PCT is a member of the calcitonin (CT) superfamily of peptides. It is a peptide of 116 amino acid with an approximate molecular weight of 14.5 kDa. It consists of three sections; the amino terminus (57 amino acids), immature calcitonin (33 amino acids) and calcitonin carboxyl-terminus peptide 1 (CCP-1) also known as katalcalcin (21 amino acids) (Huang, D. T., et al., 2018).

During inflammation, PCT is produced mainly by two alternative mechanisms; direct pathway induced by lipopolysaccharide (LPS) or other toxic metabolite from microbes and indirect pathway induced by various inflammatory mediators like IL-6, TNF- $\alpha$ , etc. (Ashitha L. et al., 2017).

PCT levels are marker for determine if a patient has an infection. This mechanism of inflammation is bacteria selectively aggregate to crystal and that bacteria are associated with an increased number of crystal-crystal agglomerations. Bacteria-crystal aggregates bind to the tubular epithelium resulting in expression of stone matrix proteins in either renal tubular epithelium or inflammatory cells. The stone matrix proteins differentiates crystalluria from progression to stone formation (Andrew L., et al., 2017).

Sodium, which is an osmotically active cation, is one of the most important electrolytes in the extracellular fluid. It is responsible for maintaining the extracellular fluid volume, and also for regulation of the membrane potential of cells. Sodium is exchanged along with potassium across cell membranes as part of active transport.

Colloid-induced AKI with morphological abnormalities of the proximal tubular cells. The tubular lesions reflect the accumulation of proximal tubular lysosomes. The tubular cells swell because they contain numerous lysosomes and endocytotic vacuoles. Furthermore, the oncotic force of colloids may induce further renal function impairment by decreasing the renal filtration pressure. The exact mechanisms of colloid-induced AKI remain incompletely elucidated, and controversy exists regarding the relative roles for morphological and functional changes that promoting acute kidney injury (Dickenmann M., et al., 2008).

The Na<sup>+</sup>/K<sup>+</sup>-ATPase helps maintain the resting potential and avail transport, and helps to regulate cellular volume as well as intracellular calcium content. Extra renal mechanisms are able to

shift K<sup>+</sup> from extra- to intracellularly, but the excretion of potassium mainly occurs in the kidneys. Renal adaptive mechanisms allow the kidneys to maintain potassium homeostasis, when glomerular filtration rate GFR is decrease, urinary potassium excretion decreases cause elevated in serum potassium. Decreasing in glomerular filtration rate is the main causes of acute kidney injury (Klevay LM, 2007).

Uric acid, which is the end product of purine metabolism in human beings, is produced by a xanthine oxidoreductase (XOR)-catalyzed reaction, and shown to have both antioxidant and pro-oxidant properties in vitro by scavenging and production of reactive oxygen species (ROS). However, its role in regulating oxidative stress under physiological conditions remains unclear, as such a reaction produces ROS (Masafumi K., et al., 2021).

The occurrence of uric acid stones has risen considerably in patients suffering from metabolic syndrome. Uric acid stones are commonly seen in patients with hyperuricosuria, Uric acid crystalluria reduces crystallization inhibitors and acts a nidus 'central location' for heterogenous calcium oxalate nucleation. Dissolved uric acid in a solution can promote precipitation crystallization which increases calcium oxalate crystal formation leading to renal calculi (Jing Pan. et al. 2018, Manish KC, 2022).

Albumin is a family of globular proteins, with molecular weight 66.5 kDa, the most common of which are the serum albumins. All the proteins of the albumin family are water-soluble, moderately soluble in concentrated salt solutions, and experience heat denaturation. Albumins are commonly found in blood plasma and differ from other blood proteins in that they are glycosylated. Albumin functions primarily as a carrier protein for steroids, fatty acids, and thyroid hormones in the blood and plays a major role in stabilizing extracellular fluid volume by contributing to oncotic pressure (known also as colloid osmotic pressure) of plasma.

Serum albumin can preserve the kidneys from toxic agents and maintain optimal oncotic pressure and kidney perfusion, Low colloid oncotic pressure as a result of hypoalbuminemia. In multivariate analysis adjusted for potential confounders, confirming an association between admission hypoalbuminemia, and increased risk of AKI (Peter B., et al., 2019).

Creatinine is a breakdown product of creatine phosphate from muscle and protein metabolism, with molecular weight

113.12 kDa, It is released at a constant rate by the body depending on muscle mass.

Creatine is synthesized primarily in the liver from the methylation of glycoylamine (guanidino acetate, synthesized in the kidney from the amino acids arginine and glycine) by S-Adenosyl methionine. It is then transported through blood to the other organs, muscle, and brain, where, through phosphorylation, it becomes the high-energy compound phosphocreatine. Creatine conversion to phosphocreatine is catalyzed by creatine kinase; spontaneous formation of creatinine occurs during the reaction (Natalie E , 2021).

Each day, 1% to 2% of muscle creatine is converted to creatinine. The conversion is nonenzymatic and irreversible. Men tend to have higher concentrations of creatinine than women because, in general, they have a greater mass of skeletal muscle. Increased dietary intake of creatine or eating a lot of protein (like meat) can increase daily creatinine excretion (Yosu L , et al. , 2021).

In pathogenesis of calcium urolithiasis, hypercalcemia and hypercalciuria are the main cause of kidney stones . Hypercalcemia and high-normal or elevated PTH levels in the context of recurrent nephrolithiasis are strongly indicative of primary hyperparathyroidism (PHPT), which is a well-recognized cause of calcium oxalate stones (Alexander R , et al. 2021 , Stephen W ,et al.,2021).

**Objective:** To estimate serum procalcitonin (PCT) in primary renal stone patients and compare it with secondary renal stone group when renal stone size  $\geq 1.5$  cm.

## SUBJECTS, MATERIAL AND METHODS

**Study Design:** This Cross-sectional Study was designed under the supervision of college of Medicine , Mustansiriya university , chemistry and biochemistry Department , and clinical data and samples were collected from March 2022 to May 2022 . Samples were taken from Al Yarmouk teaching hospital in Bagdad , urology department , under the supervision of surgical urologist , surgery department . Samples were collected from patients and non – patients attending the urology consultation and patients diagnosed with primary renal stone or recurrent renal stone , all patients has renal stone size  $\geq 1.5$  cm .

**Sampling:** Peripheral venous blood samples (about 8-10 ml) were obtained from all subjects , patients with primary and recurrent stone, (all samples were from non- fasting patients) , five to seven (5-7) milliliters were collected from the antecubital vein. Two to three (2-3) milliliters were collected without using a tourniquet for calcium test.

Blood samples were centrifuged at 3000rpm for 15 minutes (Fig 2-2), then the serum were placed into five (5) Eppendorf tubes and stored , kept frozed at  $-20\text{ }^{\circ}\text{C}$  until use in Laboratory work includes:

**Subject Groups:** A eighty eight (88) patients were registered (44 male ) and (44 female ) aged between (30-40) years. The total subjects were divided into two groups:

**Group I:** Patients with Primary Renal Stone , (Primary renal stone) called for (first incidence) , diagnosis , and first formation for a kidney stone . are diagnosed proved by X-ray technique and ultrasonography technique associated with laboratory test which verified diagnosis of renal stone disease. Include forty four (44) patients , (22 male,22 female) , aged between (30-40) years , and diagnosed by Urologist consultant .

**Group II:** Patients with Recurrent renal stone , It is the stone that recreate again (recurrent) after patient with Primary renal stone treated with extracorporeal shock wave lithotripsy (ESWL) , are diagnosed proved by X-ray technique and ultrasonography technique associated with laboratory test which verified diagnosis of renal stone disease after they treated with Extracorporeal Shock Wave Lithotripsy for patients who had previous kidney stone . Include forty four (44) patients , (22 male,22 female) , aged between (30-40) years , and diagnosed by consultant Urologist . Both groups are similar in age, weight , and sex.

**Determination of Serum Procalcitonin (PCT) level:** The procalcitonin (PCT) serum concentration was measured by enzyme-linked immune sorbent assay kit .

**A-Principle:** This kit was based on sandwich enzyme-linked immune sorbent assay technology. It is based on biotin double antibody sandwich technology to assay Human Procalcitonin(PCT). Procalcitonin(PCT) were added to wells that are pre-coated with Procalcitonin(PCT) monoclonal antibody and then incubated . After incubation, anti PCT antibodies labeled with biotin were added to unite with streptavidin-HRP, which forms the immune complex. Unbound enzymes were removed after incubation and washing, then substrate A and B were added. The solution turned to blue and changed to yellow with the effect of acid. The shades of solution and the concentration of Human Procalcitonin(PCT) were positively correlated ( Balcl C, et al. 2003).

**B-Calculation:** Figure (2.1) shows a direct relationship between the serum (PCT) concentration and the related absorption using the graphic linear exponential curve detected of standard curve. The concentration of procalcitonin (PCT) in the samples was calculated using the samples OD to standard curve as shown below:

Table 1:

Standard concentration of PCT	OD of standard 450nm
75	0.125
150	0.163
300	0.228
600	0.392
1200	0.713

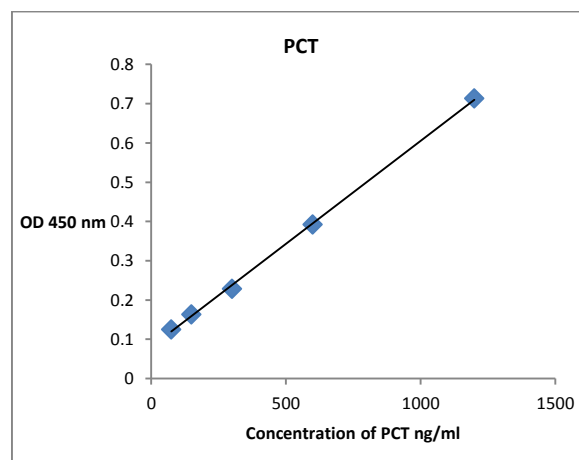
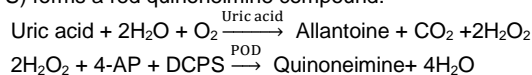


Figure 2.1: Standard curve of Serum PCT concentration.

**Determination of Serum Sodium (Na) Level:** Direct method for determining sodium concentration in biological samples by using a UV 6100 UV/VIS Spectrophotometer apparatus in the UV wavelength region. Sodium was precipitated with Mg-uranyl acetate; the uranyl ions remaining in suspension form a yellow-brown complex with thioglycolic acid. The difference between reagent blank (without precipitation of sodium) and analysis was proportional to the sodium concentration .

**Determination of Serum Uric Acid (U.A) Level:** Direct method for determining uric acid concentration in biological samples by using a UV 6100 UV/VIS Spectrophotometer apparatus in the UV wavelength region. Uric acid was oxidized by uricase to allantoin and hydrogen peroxide ( $2\text{H}_2\text{O}_2$ ), which under the influence of POD, 4-aminophenazone (4- AP) and 2-4 Dichlorophenol sulfonate (DCPS) forms a red quinoneimine compound:



The intensity of the red color formed was proportional to the uric acid concentration in the sample (Hayashi S. et al., 2000).

**Determination of Serum Albumin (ALB) Level:** Direct method for determining albumin concentration in biological samples by using a UV 6100 UV/VIS Spectrophotometer apparatus in the UV wavelength region. Albumin in the sample reacted with bromocresolgreen in acid medium forming a coloured complex that can be measured by spectrophotometry (Details found in Appendix I).

**Determination of Serum Creatinine (Cr) Level:** Direct method for determining creatinine concentration in biological samples by using a UV 6100 UV/VIS Spectrophotometer apparatus in the UV wavelength region. Creatinine in the sample was reacted with picrate in alkaline medium forming a coloured complex (Jaffé method). The complex formation rate is measured in a short period to avoid interferences. Serum and plasma samples contain proteins that react in a non-specific way; nevertheless, the results can be corrected subtracting a fixed value. The use of this correction is known as the Jaffé method compensated (Bargnoux AS, et al., 2018).

**Determination of Serum Calcium (Ca) Level:** Direct method for determining calcium concentration in biological samples by using a UV 6100 UV/VIS Spectrophotometer apparatus in the UV wavelength region. The measurement of calcium in the sample was based on formation of color complex between calcium and o-cresolphthalein in alkaline medium:



The intensity of the colour formed was proportional to the calcium concentration in the sample ( Liu, Z , et al., 2005).

**RESULTS**

**Serum Procalcitonin (PCT):** Analysis of variance for serum PCT were significant , p ≤0.0001, indicating there were significant difference in serum PCT among renal stone size in primary renal stone group and secondary renal stone group. The mean of serum PCT in male of primary renal stone group when renal stone size was ≥1.5 cm (4.020±0.650) (ng/ml) was significantly lower than male of secondary renal stone group when renal stone size was ≥1.5 cm (6.414±0.649) (ng/ml), p ≤0.0001. The mean of PCT in female of primary renal stone group when renal stone size was ≥1.5 cm (3.935±0.680) (ng/ml) was significantly lower than female of secondary renal stone group when renal stone size was ≥1.5 cm (5.318±0.953) (ng/ml), p ≤0.0001. There was significant variance p=0.008, between the mean of PCT in male and female group of secondary renal stone group when renal stone size was ≥1.5 cm . The means and standard deviations are presented in table(3-1)

Table 1: Descriptive statistics for serum PCT according to renal stone disease & renal stone size.

		Procalcitonin (PCT) (ng/ml) (176) Serum			
		Primary(44)	Recurrent (44)	P value	
Stone size (cm)	≥1.5 cm	Male	4.020±0.650	6.414±0.649	0.0001***
		Female	3.935±0.680	5.318±0.953	0.0001***
		P value	0.686	0.008#	
Data were presented as Mean±SD ,					
*Significant difference between two independent means using Students-t-test at 0.05 level.					

**Serum Sodium (Na):** Analysis of variance for serum sodium were significant , p ≤0.0001, indicating there were significant difference in serum sodium among renal stone size in primary renal stone group and secondary renal stone group. The mean of serum sodium in male of primary renal stone group when renal stone size was ≥1.5 cm (144.880±4.640) (mmol/ml) was significantly higher than male of secondary renal stone group when renal stone size was ≥1.5 cm (132.762±2.448) (mmol/ml) , p ≤0.0001. The mean of serum sodium in female of primary renal stone group when renal stone size was ≥1.5 cm (134.118±4.226) (mmol/ml) was significantly higher than female of secondary renal stone

group when renal stone size was ≥1.5 cm (133.455±2.824) (mmol/ml) , p ≤0.0001. There was significant variance p=0.011, between the mean of serum sodium in male and female group of primary renal stone group when renal stone size was ≥1.5 cm . The means and standard deviations are presented in table(3-2)

Table 2: Descriptive statistics for serum Na according to renal stone disease & renal stone size.

		Serum Na (mmol/ml) (176)			
		Primary(44)	Recurrent (44)	P value	
Stone size (cm)	≥1.5 cm	Male	144.880±4.640	132.762±2.448	0.0001***
		Female	134.118±4.226	133.455±2.824	0.0001***
		P value	0.011#	0.396	
Data were presented as Mean±SD ,					
*Significant difference between two independent means using Students-t-test at 0.05 level.					

**Serum Uric Acid:** Analysis of variance for serum uric acid were significant , p ≤0.0001, in male of secondary renal stone group when renal stone size was <1.5 . Analysis of variance for serum uric acid were significant , p ≤0.004, in male of secondary renal stone group when renal stone size was ≥1.5. The mean of uric acid in male of primary renal stone group when renal stone size was ≥1.5 cm (7.616±1.315) (mg/dL) was significantly lower than male of secondary renal stone group when renal stone size was ≥1.5 cm (8.838±1.429) (mg/dL). The mean of serum uric acid in female of primary renal stone group when renal stone size was ≥1.5 cm (7.388±1.159) (mg/dL) .The mean of female of secondary renal stone group when renal stone size was ≥1.5 cm (7.636±1.416) (mg/dL), p ≤0.0001. There was significant variance p=0.008, between the mean of serum uric acid in male and female group of secondary renal stone group when renal stone size was ≥1.5 cm . The means and standard deviations are presented in table(3-3) .

Table 3: Descriptive statistics for serum U.A according to renal stone disease & renal stone size.

		Serum uric acid (mg/dL) (176)			
		Primary(44)	Recurrent (44)	P value	
Stone size (cm)	≥1.5 cm	Male	7.616±1.315	8.838±1.429	0.004***
		Female	7.388±1.159	7.636±1.416	0.416
		P value	0.411	0.008#	
Data were presented as Mean±SD ,					
*Significant difference between two independent means using Students-t-test at 0.05 level.					

**Serum Albumin (Alb):** Analysis of variance for serum albumin were significant , p ≤0.001, indicating there were significant difference in serum albumin among renal stone size in primary renal stone group and secondary renal stone group .

The mean of serum albumin in male of primary renal stone group when renal stone size was ≥1.5 cm (33.080±3.027) (g/L) . The mean of male of secondary renal stone group when renal stone size was ≥1.5 cm (32.154±3.414) (g/L) . The mean of serum albumin in female of primary renal stone group when renal stone size was ≥1.5 cm (31.471±2.125) (g/L) . The mean of female of secondary renal stone group when renal stone size was ≥1.5 cm (32.318±3.643) (g/L) . There was significant variance p=0.032 , The means and standard deviations are presented in table(3-4) .

Table 4: Descriptive statistics for serum Albumin according to renal stone disease & renal stone size.

		Serum albumin (g/L) (176)			
		Primary(44)	Recurrent( 44)	P value	
Stone size (cm)	≥1.5 cm	Male	33.080±3.027	32.095±3.129	0.285
		Female	31.471±2.125	32.318±3.643	0.400
		P value	0.065	0.831	
Data were presented as Mean±SD					
*Significant difference between two independent means using Students-t-test at 0.05 level.					

**Serum Creatinine:** Analysis of variance serum creatinine were significant , p ≤0.0001, indicating there were significant difference

in serum creatinine among renal stone size in primary renal stone group and secondary renal stone group .

The mean of serum creatinine in male of primary renal stone group when renal stone size was  $\geq 1.5$  cm (134.160 $\pm$ 13.250) ( $\mu\text{mol/L}$ ) was significantly lower than male of secondary renal stone group when renal stone size was  $\geq 1.5$  cm (166.190 $\pm$ 17.389) ( $\mu\text{mol/L}$ ),  $p \leq 0.0001$ . The mean of serum creatinine in female of primary renal stone group when renal stone size was  $\geq 1.5$  cm (132.118 $\pm$ 12.854) ( $\mu\text{mol/L}$ ) was significantly lower than female of secondary renal stone group when renal stone size was  $\geq 1.5$  cm (167.091 $\pm$ 20.260) ( $\mu\text{mol/L}$ ) ,  $p \leq 0.0001$ . There was significant variance  $p=0.013$ , between the mean of serum creatinine in male and female group of secondary renal stone group when renal stone size was  $\geq 1.5$  cm . The means and standard deviations are presented in table(3-5) .

Tabl 5: Descriptive statistics for serum creatinine according to renal stone disease & renal stone size.

			Serum creatinine (62-106) ( $\mu\text{mol/L}$ ) (176)		
			Primary(44)	Recurrent (44)	P value
Stone size (cm)	$\geq 1.5$ cm	Male	134.160 $\pm$ 13.250	167.091 $\pm$ 20.260	0.0001***
		Female	132.118 $\pm$ 12.854	166.190 $\pm$ 17.389	0.0001***
		P value	0.622	0.013#	
-Data were presented as Mean $\pm$ SD ,					
*Significant difference between two independent means using Students-t-test at 0.05 level.					

**Serum Calcium (Ca):** Analysis of variance serum calcium were significant ,  $p \leq 0.0001$  between male primary renal stone group and secondary renal stone group The mean of serum calcium in male of primary renal stone group when renal stone size was  $\geq 1.5$  cm (4.692 $\pm$ 0.499) (mg/dL) was significantly lower than male of secondary renal stone group when renal stone size was  $\geq 1.5$  cm (5.219 $\pm$ 0.608) (mg/dL) ,  $p =0.002$ . The mean of serum calcium in female of primary renal stone group when renal stone size was  $\geq 1.5$  cm (4.635 $\pm$ 0.470) (mg/dL) was significantly lower than female of secondary renal stone group when renal stone size was  $\geq 1.5$  cm (5.295 $\pm$ 0.530) (mg/dL) ,  $p \leq 0.0001$ . The means and standard deviations are presented in table(3-6) .

Table 6: Descriptive statistics for serum calcium according to renal stone disease & renal stone size.

			Serum Ca (mg/dL) (176)		
			Primary(44)	Recurrent (44)	P value
Stone size (cm)	$\geq 1.5$ cm	Male	4.692 $\pm$ 0.499	5.219 $\pm$ 0.608	0.002***
		Female	4.635 $\pm$ 0.470	5.295 $\pm$ 0.530	0.0001***
		P value	0.713	0.662	
-Data were presented as Mean $\pm$ SD					
*Significant difference between two independent means using Students-t-test at 0.05 level.					

**DISCUSSION**

Analysis of variance for PCT were significant ,  $p \leq 0.0001$ , indicating there were significant difference in PCT among renal stone size in primary renal stone group and secondary renal stone group , secondary renal stone group was significantly higher than primary renal stone group  $p \leq 0.0001$ , see Table 3-1. This results is agreed with ( Samsudin I, et al., 2017) , and (Stephen H , et al., 2020) when they hypothesized PCT is a reliable biomarker that displays greater specificity than other proinflammatory markers, such as cytokines in identifying patients with kidney stone and aiding in the diagnosis of bacterial infections . This mechanism of inflammation is bacteria selectively aggregate to crystal and that bacteria are associated with an increased number of crystal-crystal aggregation and nucleation, Bacteria-crystal aggregates bind to the tubular epithelium resulting in expression of stone matrix proteins in either renal tubular epithelium or inflammatory cells. The stone matrix proteins differentiates crystalluria from progression to stone formation.

Analysis of variance for serum Na were significant ,  $p \leq 0.0001$ , indicating there were significant difference in serum Na

among renal stone size in primary renal stone group and recurrent renal stone group, Table (3-2) . recurrent renal stone group was significantly lower than primary renal stone group  $p \leq 0.0001$ , This results agreed with (Naoto T , et al., 2018) , and (Kiichiro F , et al . 2021) when osmotic equilibrium abnormality could induced AKI.

Analysis of variance for uric acid were significant ,  $p \leq 0.0001$ , indicating there were significant difference in uric acid among renal stone between male primary renal stone group and male recurrent renal stone group when renal stone size  $\geq 1.5$  cm Table (3-3). secondary renal stone group was significantly higher than primary renal stone group  $p \leq 0.0001$ . There is a significant  $p=0.008$  between gender in recurrent stone group when stone size  $\geq 1.5$  cm , male group is higher than female group results in Table (3-3). This finding is compatible with ( Manish KC , 2022 ) that Uric acid crystalluria reduces crystallization inhibitors and acts a nidus 'central location' for heterogenous calcium oxalate nucleation.

Analysis of variance for serum albumin were significant ,  $p \leq 0.001$ , indicating there were significant difference in albumin among renal stone size in primary renal stone group and recurrent renal stone group, Table (3-4). Male secondary renal stone group was significantly lower than male primary renal stone group  $p \leq 0.001$ . This results agreed with ( Csaba P , et al., 2012) , and (Peter B , et al ., 2019) were renal stone can cause low colloid oncotic pressure as a result of hypoalbuminemia .

Analysis of variance for creatinine were significant ,  $p \leq 0.0001$ , indicating there were significant difference in creatinine among renal stone size in primary renal stone group and recurrent renal stone group, Table (3-5) . Recurrent renal stone group was significantly higher than and a significant  $p=0.013$  between gender in secondary stone group, This finding agreed with (Vaka K. et al., 2015) , and ( Christine P , et al., 2021 ) when there is a rise in serum creatinine concentration , observed only with marked damage to functioning nephrons. serum creatinine is a reflection of glomerular filtration rate.

Analysis of variance for serum calcium were significant ,  $p \leq 0.0001$ , indicating there were significant difference in calcium among renal stone size in male primary renal stone group and male secondary renal stone group. There were significant ,  $p \leq 0.0001$ , indicating there were significant difference in calcium among renal stone size in female primary renal stone group and female recurrent renal stone group Table (3-6). Recurrent renal stone group was significantly higher than primary renal stone group, This results agreed with (Naoto T , et al ., 2018 . ) , and( Alexander R , et al. 2021).

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