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Assessment of Serum NGAL, Procalcitonin in Patients with Large Recurrent Renal Stone and Primary Renal Stone

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ABSTRACT— Neutrophil gelatinase-associated lipocalin (NGAL) is a which is extensively increased in the serum or urine of patients within a few hours after ischemia. Procalcitonin (PCT) is a peptide precursor of the hormone calcitonin, the PCT levels are marker for determine if a patient has an infection. This Cross-sectional Study was designed under the supervision of College of Medicine, Almustansiriya University, Chemistry and Biochemistry Department. A eighty five 85 patients had stone size greater or equal to 1.5 cm for (45 Male,40 Female). Serum NGAL, PCT levels in primary renal stone group was significantly lower than of secondary renal stone group. p ≤0.0001, AUC for Serum NGAL, 0.990, the cut off value was 194.50, (AUC) for serum PCT, was 1.000, cut off value was 3.950(ng/ml). Serum NGAL and serum PCT were significantly higher in recurrent renal stone than primary renal stone when renal stone size ≥1.5 cm. Considered as serum NGAL, and PCT in both of primary renal stone group and secondary renal stone group were a perfect markers for predicting Renal stone disease in patients with acute kidney injury.

KEYWORDS: Serum NGAL, lipocalin, glycoprotein

1. INTRODUCTION

Neutrophil gelatinase-associated lipocalin (NGAL) is a glycoprotein, a structural tubular marker (with a weight of 25 kDa) belonging to the lipocalin superfamily, which is extensively increased in the serum or urine of patients within a few hours after ischemia reperfusion injury. It is expressed in multiple cell types (e.g., renal, hepatic, cardiac, etc.) at relatively low, but constant levels. NGAL generally functions as a bacteriostatic agent [5]. Increases in plasma NGAL likely more readily reflect reductions in GFR. 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. 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 [2]. Serum sodium, serum uric acid, serum Albumin, serum creatinine and serum calcium are a kidney function test.

2. Materials and Methods

This Cross-sectional Study was designed under the supervision of College of Medicine, Almustansiriya University, Chemistry and Biochemistry Department, and clinical data and samples were collected from

December 2020 to March 2021. Samples were taken from Salah Aldin General Hospital in Tikrit, Urology Department, under the supervision of Surgical Urologist Doctor; Jamal Abbas, and Doctor Jamal Alsamarrie Private Hospital in Samarra, Surgical Department. Samples were collected from Hospitalized and Non – Hospitalized patients attending the Urology consultation and patients diagnosed with Secondary (recurrent) renal stone, primary renal stone, by a Urologist; All patients with Secondary Renal Stone were treated with Extracorporeal Shock Wave Lithotripsy for kidney stone.

2.1 Sampling

Peripheral venous blood samples (about 8-10 ml) were obtained from all subjects, patients with primary and secondary 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, then the serum were placed into seven (7) Eppendrof tubes and stored, kept frozed at -20 C^0 until use in Laboratory work includes:

1. Serum Neutophil Gelatinase Associated Lipocalin(NGAL).
2. Serum Procalcitonin (PCT).
3. Serum Sodium Na .
4. Serum Potassium K .
5. Serum Uric Acid.
6. Serum Albumin.
7. Serum Creatinine.
8. Serum Calcium.

2.2 Subject Groups

A eighty five 85 patients had stone size greater or equal to 1.5 cm for (45 Male,40 Female), this had subgroup according to renal stone type; A primary renal stone from 42 patients (24 male, 18 female), and a secondary renal stone from 43 patients (21 male, 22 female), aged between twenty to seventy (20-70) years, and diagnosed by consultant Urologist.

2.3 Methods

2.3.1 Determination of Serum Neutrophil gelatinase - associated lipocalin (NGAL) level

The neutrophil gelatinase-associated lipocalin (NGAL) 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 neutrophil gelatinase-associated lipocalin(NGAL). Neutrophil gelatinase-associated lipocalin(NGAL) were added to wells that are pre-coated with neutrophil gelatinase-associated lipocalin(NGAL) monoclonal antibody and then incubated. After incubation, anti NGAL 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 was turned to blue and change to yellow with the effect of acid. The shades of solution and the concentration of Human neutrophil gelatinase-associated lipocalin(NGAL) were positively correlated.

2.3.2 Determination of Serum Procalcitonin (PCT) level

The procalcitonin (PCT) serum concentration was measured by enzyme-linked immune sorbent assay kit.

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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.

2.3.3 Objective

To estimate serum neutrophil gelatinase associated lipocalin (NGAL), serum procalcitonin (PCT), serum Na, serum Albumin, serum uric acid, serum creatinine, and serum calcium, in large size primary renal stone patients and compare it with large size secondary renal stone group.

3. Results

The mean of NGAL in male of primary renal stone group when renal stone size was ≥1.5 cm (189.480±4.283) (ng/ml) was significantly lower than male of secondary renal stone group when renal stone size was ≥1.5 cm (213.095±9.137) (ng/ml), p ≤0.0001. The mean of NGAL in female of primary renal stone group when renal stone size was ≥1.5 cm (188.824±3.283) (ng/ml) was significantly lower than female of secondary renal stone group when renal stone size was ≥1.5 cm (209.909±9.159) (ng/ml), *p* ≤0.0001. The means and standard deviations are presented in table (3-1).

Table (3-1): Descriptive statistics for NGAL according to renal stone disease

			SerumNeutrophil Gelatinase Associated Lipocalin (NGAL) (ng/ml) (176)		
			Primary(88)	Secondary(88)	P value
	=>1.5 cm	Male (24,21)	189.480±4.283	213.095±9.137	**0.0001#**
		Female (18,22)	188.824±3.283	209.909±9.159	**0.0001#**
		P value	0.597	0.260	
-Data were presented as Mean±SD					
#Significant difference between two independent means using Students-t-test at 0.05 level.					

The mean of PCT in male of primary renal stone group when renal stone size was ≥1.5 cm (3.020±0.650) (ng/ml) was significantly lower than male of secondary renal stone group when renal stone size was ≥1.5 cm (6.214±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 (2.935±0.680) (ng/ml) was significantly lower than female of secondary renal stone group when renal stone size was ≥1.5 cm (5.518±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 (3-2).

Table (3-2): Descriptive statistics for PCT according to renal stone disease

			Procalcitonin (PCT) (ng/ml) (176) Serum		
			Primary(88)	Secondary(88)	P value
	=>1.5 cm	Male (24,21)	3.020±0.650	6.214±0.649	**0.0001#**
		Female (18,22)	2.935±0.680	5.518±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.					

The mean of sodium in male of primary renal stone group when renal stone size was ≥1.5 cm (140.880±4.640) (mmol/ml) was significantly higher than male of secondary renal stone group when renal stone size was ≥1.5 cm (131.762±2.448) (mmol/ml), $p \leq 0.0001$. The mean of sodium in female of primary renal stone group when renal stone size was ≥1.5 cm (137.118±4.226) (mmol/ml) was significantly higher than female of secondary renal stone group when renal stone size was ≥1.5 cm (132.455±2.824) (mmol/ml), $p \leq 0.0001$. There was significant variance $p=0.011$, between the mean of 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 (3-3).

Table (3-3): Descriptive statistics for Na according to renal stone disease.

			Serum Na (mmol/ml) (176)		
			Primary(88)	Secondary(88)	P value
	=>1.5 cm	Male (24,21)	140.880±4.640	131.762±2.448	**0.0001#**
		Female (18,22)	137.118±4.226	132.455±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.					

Analysis of variance for uric acid were significant, $p \leq 0.004$, 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 (7.516±1.315) (mg/dL) was significantly lower than male of secondary renal stone group when renal stone size was ≥1.5 cm (8.738±1.429) (mg/dL). The mean of uric acid in female of primary renal stone group when renal stone size was ≥1.5 cm (7.188±1.159) (mg/dL). The mean of female of secondary renal stone group when renal stone size was ≥1.5 cm (7.536±1.416) (mg/dL), $p \leq 0.0001$. There was significant variance $p=0.008$, between the mean of 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

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table (3-4).

Table (3-4): Descriptive statistics for U.A according to renal stone disease

			Serum uric acid (mg/dL) (176)		
			Primary(88)	Secondary(88)	P value
	=>1.5 cm	Male (24,21)	7.516±1.315	8.738±1.429	**0.004#**
		Female (18,22)	7.188±1.159	7.536±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.					

The mean of 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 albumin in female of primary renal stone group when renal stone size was ≥1.5 cm (37.929±3.792) (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, between the mean of albumin 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 (3-5).

Table (3-5): Descriptive statistics for Albumin according to renal stone disease.

			Serum albumin (g/L) (176)		
			Primary(88)	Secondary(88)	P value
	=>1.5 cm	Male (24,21)	33.080±3.027	32.095±3.129	0.285
		Female (18,22)	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.					

The mean of creatinine in male of primary renal stone group when renal stone size was ≥1.5 cm (154.160±13.250) (µmol/L) was significantly lower than male of secondary renal stone group when renal stone size was ≥1.5 cm (186.190±17.389) (µmol/L), p ≤0.0001. The mean of creatinine in female of primary renal stone group when renal stone size was ≥1.5 cm (152.118±12.854) (µmol/L) was significantly lower than female of secondary renal stone group when renal stone size was ≥1.5 cm (201.091±20.260) (µmol/L), p ≤0.0001. There was significant variance p=0.013, between the mean of creatinine 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 (3-6).

Table (3-6): Descriptive statistics for creatinine according to renal stone disease & renal stone size.

			Serum creatinine (62-106) (µmol/L) (176)		
			Primary(88)	Secondary(88)	P value
	=>1.5 cm	Male (24,21)	154.160±13.250	201.091±20.260	**0.0001#**
		Female (18,22)	152.118±12.854	186.190±17.389	**0.0001#**
		P value	0.622	**0.013#**	
-Data were presented as Mean±SD					
#Significant difference between two independent means using Students-t-test at 0.05 level.					

The mean of calcium in male of primary renal stone group when renal stone size was ≥1.5 cm (4.692±0.499) (mg/dL) was significantly lower than male of secondary renal stone group when renal stone size was ≥1.5 cm (5.219±0.608) (mg/dL), p =0.002. The mean of calcium in female of primary renal stone group when renal stone size was ≥1.5 cm (4.635±0.470) (mg/dL) was significantly lower than female of secondary renal stone group when renal stone size was ≥1.5 cm (5.295±0.530) (mg/dL), p ≤0.0001. The means and standard deviations are presented in table (3-7).

Table (3-7): Descriptive statistics for calcium according to renal stone disease & renal stone size.

			Serum Ca (mg/dL) (176)		
			Primary(88)	Secondary(88)	P value
	=>1.5 cm	Male (24,21)	4.692±0.499	5.219±0.608	**0.002#**
		Female (18,22)	4.635±0.470	5.295±0.530	**0.0001#**
		P value	0.713	0.662	
-Data were presented as Mean±SD					
#Significant difference between two independent means using Students-t-test at 0.05 level.					

Table (3-8): ROC analysis criteria of selected markers as differentiating between primary renal stone group and secondary renal stone group

Test Result Variables	**Area Under the Curve (AUC)**	**Std. Error**	**P value**	**95% Confidence Interval**	
				Lower Bound	**Upper Bound**
Neutrophil Gelatinase Associated Lipocalin (NGAL) (ng/ml)	**0.990**	**0.009**	**0.0001**	**0.971**	**1.000**

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Procalcitonin (PCT) (ng/ml)	**1.000**	**0.001**	**0.0001**	**-**	**-**

4. Discussion

Analysis of variance for serum NGAL were significant, $p \leq 0.0001$, indicating there were significant difference in serum NGAL when renal stone size ≥1.5 cm, in primary renal stone group and secondary renal stone group. Increasing reactive oxygen species in response to oxalate and Calcium oxalate crystals are in part produced with the involvement of NADPH oxidase through the activation of the rennin angiotensin system (RAS). Reduction of angiotensin production, by inhibiting the angiotensin converting enzyme as well as blocking the angiotensin receptor, increased renin expression, reduced osteopontin (OPN) expression. Role of OPN in nephrolithiasis is to inhibit Calcium oxalate crystallization, aggregation and crystal attachment, thus promote stone formation. This agreed with what [8], [9] when NGAL found in promoting kidney stone disease.

Analysis of variance for PCT were significant, $p \leq 0.0001$, indicating there were significant difference in PCT when renal stone size ≥1.5 cm in primary renal stone group and secondary renal stone group. This results is agreed with [11], [10] 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 when renal stone size ≥1.5 cm in primary renal stone group and secondary renal stone group.

This results agreed with [6], [3] when osmotic equilibrium abnormality could induced AKI uric acid were significant, $p \leq 0.0001$, indicating there were significant difference in uric acid when renal stone size ≥1.5 cm in primary renal stone group and secondary renal stone group. This finding is compatible with [4], that Uric acid crystalluria reduces crystallization inhibitors and acts a nidus 'central location' for heterogenous calcium oxalate nucleation. serum albumin were significant, $p \leq 0.001$, indicating there were significant difference in albumin when renal stone size ≥1.5 cm in primary renal stone group and secondary renal stone group. This results agreed with [7], [13] were renal stone can cause low colloid oncotic pressure as a result *of* hypoalbuminemia. Also creatinine were significant, $p \leq 0.0001$ in these groups. This finding agreed with [14], [12] 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. Serum calcium were significant, $p \leq 0.0001$, indicating there were significant difference in calcium when renal stone size ≥1.5 cm in primary renal stone group and secondary renal stone group. This results agreed with [6], [1]. Elevated Calcium concentration is a main cause for calcium oxalate stone. The area under the curve (AUC) for was 0.990, the cut off value was 194.50, in comparison between primary renal stone group and secondary renal stone group, primary renal stone group has NGAL level lower than 194.50 (ng/ml), secondary renal stone group has NGAL level higher than 194.50 (ng/ml). area under the curve (AUC) for serum PCT, was 1.000, cut off value was 3.950(ng/ml), in comparison between primary renal stone group and secondary renal stone group, primary renal stone group has serum PCT level lower than 3.950 (ng/ml), secondary renal stone group has PCT level higher than 3.950 (ng/ml). Considered as serum NGAL, and PCT in both of primary renal stone group and secondary renal stone group were a perfect markers for predicting Renal stone disease in patients with acute kidney injury.

5. Conclusion

Serum NGAL and serum PCT were significantly higher in recurrent renal stone than primary renal stone when renal stone size ≥1.5 cm, When the renal stone size ≥1.5 there is an increased in all the study markers level markers in the secondary (recurrent) renal group than primary group, except for serum sodium and albumin.

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