Evaluation of NGAL biomarker and some biochemical parameter in patient with Renal disorder in Ramadi city

Abstract :

This study was designed to determine the effect or the relationship between Neutrophil gelatinase-associated lipocalin (NGAL) and some kidney function parameter in kidney disorder patientsto detect the level of serum markers in kidney disorder patients(Acute glomerular nephritis, Renal Calculi, and Acute renal failure in Ramadi City. 120 male and female samples participated, the present study was divided into three groups of 90 patients (30Acute glomerular nephritis,30Renal Calculi, and 30 Acute renal failure) and 30 healthy control individuals.The serum NGAL wase measured using an ELISA technique. Kidney function test (serum Creatinine and serum Urea, HB,WBC) were also measuredby quantitative method.

The result showed,low significant($p>0.001$) differences in hemoglobin and WBC percent in Acute Renal Failure group compare with other groups,andwhen compare between the studied groups the results showed high significant($p<0.001$) differences in Urea and creatinine concentration in Acute Renal Failure group compare with (AGN and RC) group.

On the other hand the result showed high significant($p<0.001$) differences in level of NGASL in Acute Renal Failure group compare with others groups.

The correlation between parameter showed higher negative correlation between Hb with urea, creatinine, and NGASL with ($r=-0.571$, -0.508, and -0.463) respectively. In addition to higher correlation between urea and creatinine with NGASL ($r=0.670$, 0.406) respectively.So can be considered NGAL a biomarker for Kidney injury or kidney disorder . That may be indicate the NGASL effect of renal disease and may be used as predict parameters for kidney diseases.

Key word:Renal disorder, Neutrophil Gelatinase-Associated Lipocalin(NGASL), urea, creatinine .

1. Introduction

Kidney disorder are considered one of the very common problems in Iraq and are considered a chronic disease that may sometimes lead to death in some people, There are routine tests used in medical laboratories to determine kidney disease problems, but these tests may not be very suitable for identifying kidney problems, so in this research we used tests New and very accurate, it is used in the initial diagnosis of kidney problems and determining its functions very accurately **(**Cai *et al*., 2010)

Kidney disease often starts slowly and develops without symptoms over a number of years, so CKD may not be detected until it has progressed to the point where your kidney function is quite low**.** Fortunately, most people do not progress to end-stage kidney disease, especially if they are diagnosed early and are able to take steps to preserve their remaining kidney function**(**Flo *et al*., 2004).

Neutrophil gelatinase-associated lipocalin NGAL is a 25 kDa protein of the lipocalin family. Lipocalin proteins are composed of 8 βstrands that form a β-barrel enclosing a calyx (Flower *et al*., 2000). This NGAL structure was illustrated by crystallography (Goetz *et al*., 2002). The calyx binds and transports small molecules. NGAL was originally identified in neutrophils, but it is also expressed in kidney, liver and epithelial cells in response to various pathologic states, such as inflammation, infection, intoxication, ischemia, acute kidney injury and neoplastic transformation.

Kidney Disease

The kidneys are two bean-shaped organs. Each kidney is about the size of a fist. Your kidneys filter extra water and wastes out of your blood and make urine. Kidney disease means your kidneys are damaged and can't filter blood the way they should**(**Perry *et al* .,2010).

Kidney diseases can affect your body's ability to clean your blood, filter extra water out of your blood, and help control your blood pressure. It can also affect red blood cell production and vitamin D3 metabolism needed for bone health. You're born with two kidneys. They're on either side of your spine, just above your waist **(**Kashani *et al*.,2013).

Types of Kidney Disease

Chronic kidney disease (CKD)

Chronic kidney disease (CKD) is a clinical syndrome secondary to the definitive change in function and/or structure of the kidney and is characterized by its irreversibility and slow and progressive evolution. Another important aspect is the pathology represents a higher risk of complications and mortality, especially cardiovascular-related(National Kidney Foundation ,2002) .

In addition to being highly prevalent, CKD is associated with a higher risk of cardiovascular disease, severity, and death. In fact, global data from 2013 showed that the reduction in GFR was associated with 4% of deaths worldwide, i.e., 2.2 million deaths. More than half of those deaths were due to cardiovascular causes, while 0.96 million were related to end-stage renal disease(Thomas *etal*.,2017) (. The aforementioned SBN census found a gross annual mortality rate of 19.9% on dialysis.

Glomerulonephritis

Glomerulonephritis (gloe-MER-u-loe-nuh-FRY-tis) is inflammation of the tiny filters in the kidneys (glomeruli)**(**Marinho *et al*., 2017) . The excess fluid and waste that glomeruli (gloe-MER-u-lie) remove from the bloodstream exit the body as urine. Glomerulonephritis can come on suddenly (acute) or gradually (chronic)**(**Coresh *et al*., 2007).

Severe or prolonged inflammation associated with glomerulonephritis can damage the kidneys. Treatment depends on the type of glomerulonephritis you have(Vassalotti *et al*., 2016)

Polycystic kidney disease (PKD)

Polycystic kidney disease (PKD) is an inherited disorder in which clusters of cysts develop primarily within your kidneys, causing your kidneys to enlarge and lose function over time. Cysts are noncancerous round sacs containing fluid**(**MATTIX HJ *et al*., 2002) . The cysts vary in size, and they can grow very large. Having many cysts or large cysts can damage your kidneys (JOHNSON *et al* ., 2004) .

Acute kidney injury (AKI)

Acute kidney injury (AKI) is a sudden and recent reduction in the level of kidney function. Doctors usually say AKI is severe when the kidney function, measured by blood tests **(**Khwaja, A. KDIGO. 2012)**.** has dropped by one half (50%).

Acute kidney injury often gets better in a few days or weeks. It is often caused by 'stress' on the kidney from problems elsewhere in the body (Soni, *et al*. 2010**)** , rather than diseases starting in the kidney. However, if you are identified as at risk then it is important you seek specialist assessment and treatment to ensure the issue does not progress **(**Venge,2018**)**.

Kidney stones

Kidney stones(also called renal calculi, nephrolithiasis or urolithiasis)prevention of renal stone recurrence remains to be a serious problem in human health (Mikawlrawng*etal*.,2014). prevention of stone recurrence requires better understanding of the mechanisms involved in stone formation (Khan *etal*.,2016). Kidney stones have been associated with an increased risk of chronic kidney diseases (Sigurjonsdottir *etal*.,2015), end-stage renal failure (Mikawlrawng etal.,2014, El-Zoghby *etal*.,2012), cardiovascular diseases [Rule etal.,2010, Worcester and Coe 2008], diabetes, and hypertension (Marinho *etal*.,2017). It has been suggested that kidney stone may be a systemic disorder linked to the metabolic syndrome. Nephrolithiasis is responsible for 2 to 3% of end-stage renal cases if it is associated with nephrocalcinosis (Courbebaisse*etal*.,2017)

A kidney stone usually will not cause symptoms until it moves around within the kidney or passes into one of the ureters (Moro*et al*., 2010) .

Neutrophil gelatinase-associated lipocalin (NGAL)

Neutrophil gelatinase-associated lipocalin (NGAL), a 25 kDa protein produced by injured nephron epithelia, is one of the most promising new markers of renal epithelial injury (Bouchard *et al*., 2016). In contrast to serum creatinine and urinary output, which are the measures of kidney function, NGAL is specifically induced in the damaged nephron and then released into blood and urine, where it can be readily measured (Independent Hospital Pricing Authority (AU). 2015). Clinical studies indicate that NGAL, unlike creatinine, is a marker responsive to tissue stress and nephron injury, but less so to adaptive hemodynamic responses. In certain clinical settings, NGAL is an earlier marker compared with serum creatinine (Park, *et al*.,2005) .

Neutrophil gelatinase-associated lipocalin (NGAL) is protein bound to gelatinase, and it was first described in neutrophils. Circulating NGAL is normally reabsorbed at the level of the proximal tubule, and, after ischemia, NGAL is secreted in the thick ascending limb and found in the urine (National Institute for Health and Clinical Excellence.2008).

NGAL may be increased in patients with infections. Thus, its value for diagnosing early ARF in complicated, septic patients may be limited. Urinary NGAL measurement has recently become commercially available**(**KDIGO clinical practice guideline for the diagnosis, 2009).

Neutrophil gelatinase-associated lipocalin (NGAL) is an independent biologic marker able to detect earlier AKI than serum creatinine . In fact serum creatinine is a marker of kidney function, whereas NGAL is a marker of kidney injury. Moreover, NGAL levels are useful to quantify the degree of tubular damage to establish the stratification of AKI (National Institute for Health and Care Excellence. 2013) . The limitations of using NGAL in ED seem to be related to the false-positive levels in septic patients or in chronic kidney diseases (Australian Commission on Safety and Quality in Health Care. 2017).

Aim of study:

To investigate the role of plasma NGAL as an early biomarker of renal kidney injury patient inRamadi city /Iraq.

Material and method

Sample study

Blood samples were collected from120 patient in hospitals in the Ramadicityfrom people suffering of kidney problems. The samples were distributed as follows: 30 samples for healthy people (10 men and 10 women), 30 samples for people who's suffering from acute nephronitis, 30 samples for people with kidney stones. And 30 samples for people with acute kidney failure,the samples were collected for the period from January 1 to February 25, 2024.

A venous blood sample was collected, a total of blood (3-5 ml) from each patients with kidney problems and it was divided into two tubes (2ml), for the whole blood sample was dispensed in tube containing ethylene di amine tetra acetic acid into (an EDTA-Tube) was used for estimation CBC. The second sample part of blood (2ml) was put into Gel tube, Allow serum to clot for 10-20 minutes at room temperature. to measure the concentration **NGASL** biomarkerand concentration of serum urea , creatinine ,hemoglobin and WBC.

Statistical Analysis

SAS (2012) version 9 was used for the statistical analysis . Pprogram was used to investigate the effect of data differences between groups. All results have been expressed as mean ± standard error (M ± SD). Differences between groups have analyzed using an analysis of variance (ANOVA), Least significant difference (LSD) test. Pearson correlation coefficient (r) was calculated to check the interdependence of variables, and chi-square has been used to test the significant difference between groups. ($P<0.05$) has been regarded as statistically significant .Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability in this study.

Results and discussion

1-Comparative between study groups to determine the Effect of NGASL and some parameter

Table No.1 and Figures (1,2,3,4)clarify the mean ± SD between all study group(AGN, RC, ARF, and controls group) used ANOVA test . The results showed low significant($p>0.001$) differences in hemoglobin percent between all study groups (AGN, RC, ARF) compared with controls group, and when compare between study group the result showed low significant($p>0.001$) differences in hemoglobin percent and WBC percent in Acute Renal Failure group compare with other groups. And when compare between study groups the result showed high significant($p<0.001$) differences in Urea and creatinine concentration in all study group compared with control group, and when compare between the study groups the results showed high significant($p<0.001$) differences in Urea and creatinine concentration in Acute Renal Failure group compare with (AGN and RC) . On the other hand the result showed high significant($p<0.001$) differences in level of NGASL in all study groups ,and when compare between the three study groups the result showed high significant($p<0.001$) differences in Acute Renal Failure group compare with others groups. That may be indicate the NGASL effect of renal disease and may be used as predict parameters for kidney diseases.

Table No.(1) Compare between study groups to determine the Effect of NGASL and someparameter

Variables	Mean ± SD				LSD value	P-value
	Controls	Acute glomerulonephritis	Renal calculi	Acute Renal failure		
Hemoglobin (%)	14.2 ± 1.4 a	10.2 ± 1.8 b	11.9 ± 2.7 b	9.1 ± 1.3 b	1.794 **	0.001
WBC (cell/mm^3)	7.96 ± 2.14 c	11.2 ± 4.4 b	14.1 ± 3.3 a	5.95 ± 2.66 c	1.802 **	0.001
Urea (mg/dl)	32.1 ± 7.3 d	75.2 ± 33.8 b	51.9 ± 15.9 c	101.5 ± 48.6 a	17.54 **	0.001
Creatinine (mg/dl)	0.9 ± 0.16 b	1.69 ± 0.93 b	1.35 ± 0.53 b	3.65 ± 1.22 a	0.891 **	0.001
NGASL (ng/ml)	112.4 ± 65.5 d	289.8 ± 86.8 c	665.8 ± 119.1 b	912.5 ± 239.3 a	106.45 **	0.001
Means having with the different letters in same row differed significantly. ** ($P\leq0.01$).						

Figure for Anova to descripe differences between Urea concentration in study groups

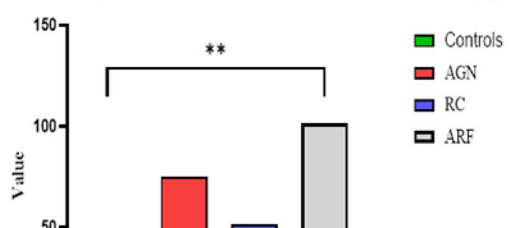
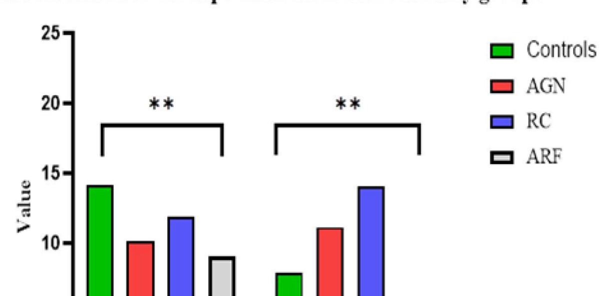


Figure for Anova to descripe differences between study groups



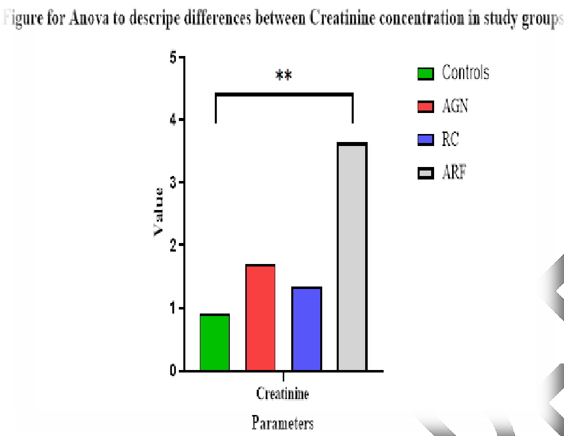


Fig 3. Anova to descripe differences between creatinine concentration in study groups

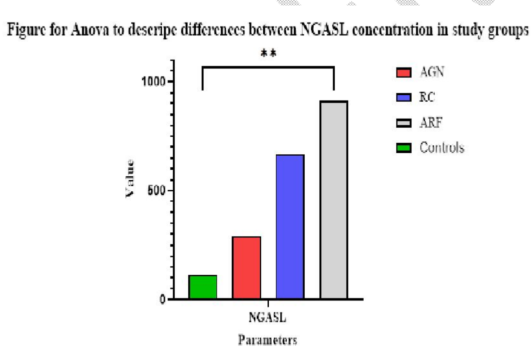


Figure (4) Anova to descripe differences between NGASL concentration in study groups

Discussion

Thepresent study assessed the hematological parameters and comorbidities among hospitalized CKD patients. We observed some blood parameters altered in CKD patients. Among the parameters, we found a decreased Hb, RBC,WBC. Also, we noticed the degree of alterations directly associated with the severity of CKD patients.this study agree with Alemu *et al*., (2021) who reported that A cross-sectional study among 251 CKD patients in Northwest Ethiopia reported that the prevalence of anemia was 64% which varies from 44.8% to 93.8% with increasing stages of CKD .

Another study showed that 19.5% of CKD patients developed severe anemia, and 25% of anemia-affected CKD patients developed anemia even after receiving therapy.(Adera *et al*., 2019) Also, study of George *et al*., (2018) showed that a cross-sectional study among 1564 mixed ancestry South African patients showed lowered RBC and Hb levels in the case of CKD participants A prevalence of anemia ranged from 37.2% (stage 3) to 82.4% (stage 4-5). There is also the prevalence of anemia even after hemodialysis in the case of a patient with CKD.

The reduced Hb levels may be due to iron-deficiency anemia caused by CKD.(Batchelor *et al*., 2020) Another potential mechanism of developing anemia in CKD patients is the reduced RBC lifespan as the production of erythropoietin reduces in CKD with the progression of the staging.(Babitt *et al*., 2012).

And when compare between study groups the result showed high significant($p<0.001$) differences in Urea and creatinine concentration in all study group compared with control group, and when compare between the study groups the results showed high significant($p<0.001$) differences in Urea and creatinine concentration in Acute Renal Failure group compare with (AGN and RC) , this study agree with Al-Sabah*etal*(2024) who showedcreatinine among AKI patients stages and the control group found highly significant differences ($P\leq0.001$) between stage 3 and control, stage 3 and stage 1, and stage 3 and stage 2. Also, a high significant difference in $P\leq0.001$ between all patients and the control group related to that developed AKI, is defined as an increase in serum creatinine, and this is because of creatinine elimination by the kidney and when the kidney damage causes an increase in serum creatinine. Nevertheless, serum creatinine levels are used to diagnose and stratify AKI, which agrees with the (Nehomar,2020) and urea among AKI patients stages and the control group found highly significant differences ($P\leq0.001$) among stage 1, stage 2, and stage 3 with control, between stage 1 and stage 3, and between stage 2 and stage 3. Also, a high significant difference in $P\leq0.001$ between all patients and the control group related to urea is it product of proteins and nitrogen metabolism. urea is the most abundant substance in the blood of uremic people (Noman Salman,2022) in patients with heart failure, decreased cardiac output and insufficient arterial filling lead to the release of the sympathetic nervous system (SNS) and renin-angiotensin-aldosterone system (RAAS), and increased sodium reabsorption in proximal renal tubules, resulting in increased urea concentration.

The current result shows a trend increasing in level of NGAL level of NGASL in all study groups ,and when compare between the three study groups the result showed high significant($p<0.001$) differences in Acute Renal Failure group compare with others groups, this study agree with(Al-Sabah*etal*.,2024) shows NGAL among AKI patients' stages 1 and the control group found highly significant differences ($P\leq0.01$) among all stages with control. Also, a high significant difference in $P\leq0.01$ between all patients and the control group. NGAL was first identified in neutrophil granules; it is nearly entirely reabsorbed in the proximal tubule, and increased levels can be a sign of proximal tubular damage (Srisawat and Kellum ,2020). This pro-inflammatory mediator is generated as a result of tissue damage and acts as a biomarker for the early detection of kidney injury. All nephron segments have the potential to be harmed after an ischemia event, however the proximal tubular cells are typically the most injured. When AKI is present, the distal tubule and Henle's loop can produce 1000 times more NGAL, and this agrees with (Menez and Parikh ,2019)

and disagrees with (Capelli *etal*.,2020), which demonstrated that NGAL showed a slight increase in their study of kidney injury.
On the basis of these unique properties, recent study have validated the reliability of NGAL as a specific, sensible, and early predictor of AKI after cardiac surgery, contrast administration, septic shock, and even renal transplantation (Ling *et al*., 2008). In current study, NGAL was measured in a cohort of patients affected by nonadvanced CKD with stable renal function. Interestingly, apart from the already cited predictive value, a strict, independent, and inverse correlation with estimated GFR was described for both sNGAL and uNGAL, suggesting that under these particular conditions this protein may also represent a surrogate index of residual renal function, similar to what has previously been described elsewhere **(**Bolignano*et al*., 2008).

2-Correlation between parameters of all study groups

Table No.2 Showed the correlation between all study groups(Acute glomerular nephritis AGN, acute renal failure ARF, Renal calculi RC)in some studied parameters (Hb, WBC, Urea, Creatinine, NGASL)
The result showed high(r= -0.571, p≤0.001) negative correlation between urea and Hemoglobin. Also, the result of correlation test showed high(r=-0.508, -0.463, p≤0.001) negative correlation between hemoglobin percent with creatinine and NGASL respectively.
On the other hand , the result found that high(r= -0.253 , p≤0.001) negative correlation between WBC and creatinine concentration with
The result showed that high(r= 0.670, 0.406, p≤0.05)positive correlation between urea with creatinine and NGASL respectively.
Creatinine concentration also showed high(r=0.573 , p≤0.0001) positive correlation with NGASL.
This result indicate that decrease in hemoglobin percent lead to increase in (Urea, creatinine, and NGASL) and this study approchesto study of.(Segall *et al*.,2014) who showed patients with CKD might suffer from different types of cardiac diseases, decreased Hb and RBC levels might cause anemia in CKD patients. Also, noticed negative associations between Hb and RBC levels with the staging of CKD patients. Reduced renal function results in decreased erythropoietin production, which results in decreased Hb synthesis and a decrease in the total number of RBCs.(Kutuby*et al*.,2015) The patient develops anemia because of this significant decrease in RBC.40 Iron and erythropoietin produce RBC in the bone marrow. (Batchelor *et al*., 2020)
This result indicate that increasing in urea concentration lead to increase in creatinine and NGASL concentrationNevertheless, serum creatinine levels are used to diagnose and stratify AKI, this study which agrees with the (Nehomar, 2020) and urea among AKI patients stages and the control group found highly significant differences (P≤0.001) among stage 1, stage 2, and stage 3 with control, between stage 1 and stage 3, and between stage 2 and stage 3. Also, a high significant difference in P≤0.001 between all patients and the control group related to urea is it product of proteins and nitrogen metabolism, urea is the most abundant substance in the blood of uremic people (Noman Salman*etal*.,2022).
The result indicate that creatinine and NGASL may be used together as ideal biomarker for identify renal disease. This pro-inflammatory mediator is generated as a result of tissue damage

and acts as a biomarker for the early detection of kidney injury. All nephron segments have the potential to be harmed after an ischemia event, however the proximal tubular cells are typically the most injured. When AKI is present, the distal tubule and Henle's loop can produce 1000 times more NGAL, and this disagrees with (Capelli *etal.*,2020), which demonstrated that NGAL showed a slight increase in their study of kidney injury.

Table No.2: Correlation between parameters of all study groups

Variables	Correlation	Hb	WBC	Urea	Creatinine	NGASL
Hb	R	1	0.105	**-0.571****	**-0.508****	**-0.463****
	Sig.		0.176	**0.0001**	**0.0001**	**0.0001**
WBC	R		1	-0.131	**-0.253***	-0.104
	Sig.			0.123	**0.012**	0.179
Urea	R			1	**0.670****	**0.406****
	Sig.				**0.0001**	**0.0001**
Creatinine	R				1	**0.573****
	Sig.					**0.0001**
NGASL	R					1
	Sig.					

**Correlation is significant at the 0.01 level (1-tailed)
*Correlation is significant at the 0.05 level (1-tailed)

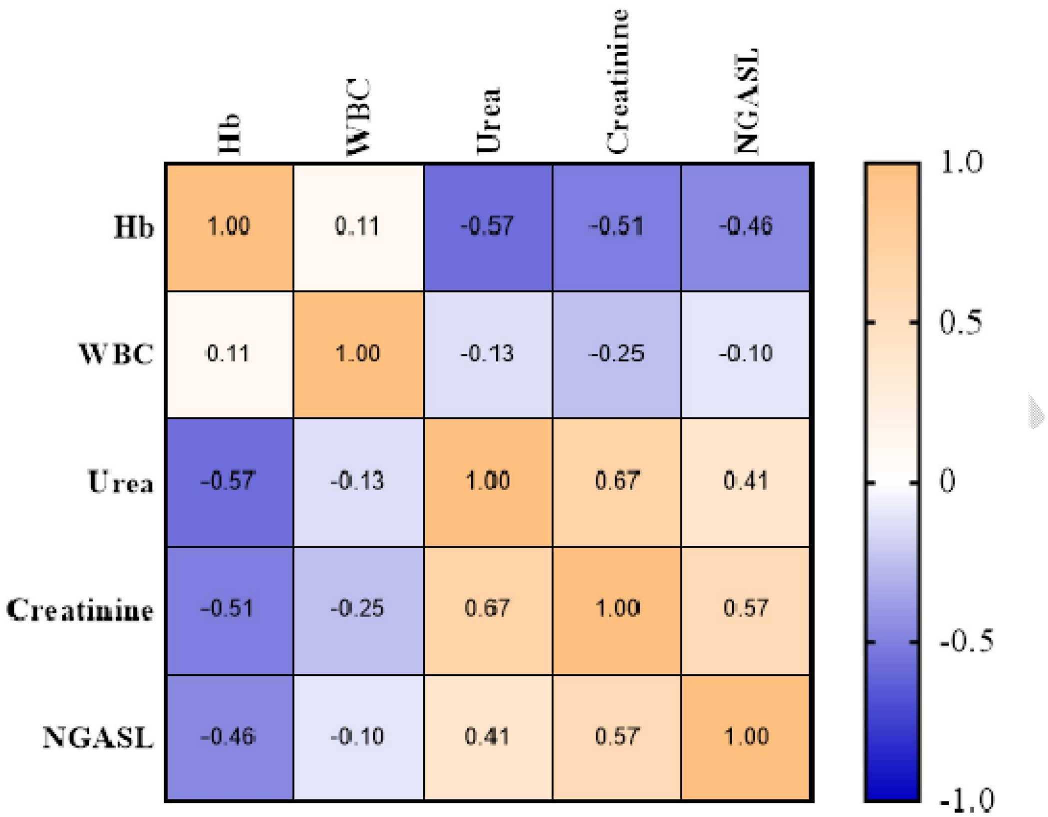


Figure (5) Correlation mapbetween parameters of all study groups

Conclusion

1. An increase in the protein Neutrophil gelatinase associated lipocalin was observed in Renal failure patients patients compared to acute nephritis patients and Renal calculi patients. And decrease in the level of hemoglobin and red blood cells was observed in patients with kidney failure compared to other groups. The study summary proved that the protein neutrophil gelatinase associated lipocalin (NGAL) is more specific in diagnosing kidney problems than the urea and creatinine tests for all groups

List of Abbreviations:

(NGAL) -Neutrophil gelatinase-associated lipocalin

(AGN) -Acute glomerular nephritis,

(RC)-Renal Calculi,

(ARF)- Acute renal failure

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