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The Correlation between Serum Neutrophil Gelatinase-Associated Lipocalin and Iron Status in Predialysis Chronic Kidney Disease

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Abstract
Background: Chronic Kidney disease "CKD" is a chronic and progressive major health problem affecting a large proportion of the population and it is threatening to reach epidemic proportions over the next decade. A normochromic, normocytic anemia usually accompanies progressive CKD patients, with a reported prevalence of 47.7% among pre-dialysis patients. Human neutrophil gelatinase-associated lipocalin (NGAL, also known as lipocalin 2, siderocalin or 24p3) was originally isolated from the supernatant of activated neutrophils and identified as a polypeptide covalently bound to gelatinase. The aim of the study was to evaluate role of serum NGAL as biomarker of iron deficiency in CKD patients. **Methods:** 72 paricipants divided into 2 groups, 36 predialysis CKD patients on conservative treatment (CKD group) and 36 non-CKD participants as a control group. All participants in this study were subjected to full history taken, medical examination and laboratory investigation including CBC, Kidney functions, liver function, "S. Iron, and Serum NGAL) were measured.**Results:** There was no significant difference between the two groups of the study as regard age, gender and body mass index (BMI) distributions. Regarding kidney functions (Blood urea and serum creatinine) and eGFR distributions, our results showed that there was a statistical significant difference between the studied groups. Serum iron were found to be higher in the control group than in the CKD group. Regarding serum NGAL level, our result showed that serum NGLA was higher in CKD group than in the non-CKD group with a statistical significance. **Conclusion:** Human neutrophil gelatinase-associated lipocalin is significantly higher when compared with controls and this means that it has an important rule iron metabolism in those patients. Serum NGAL can be a good biomarker for iron status in CKD patients.
Key words: NGAL- biomarker- CKD patients.

Introduction:

Chronic Kidney disease "CKD" is a chronic and progressive major health problem affecting a large proportion of the population [(1)] and it is threatening to reach epidemic proportions over the next decade [(2)]. It increases the risk for many adverse health outcomes including cardiovascular disease, end-stage renal disease (ESRD), and mortality [(3)].

A normochromic, normocytic anemia usually accompanies progressive CKD patients, with a reported prevalence of 47.7% among pre-dialysis patients [(4)]. It is one of the Common laboratory and clinical findings of chronic kidney diseases (CKD). Also it is a major and

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common complication of this disease that contributes to its progression, cardiovascular events and high morbidity and mortality in CKD patients [(5)].
Human neutrophil gelatinase-associated lipocalin (NGAL, also known as lipocalin 2, siderocalin or 24p3) was originally isolated from the supernatant of activated neutrophils and identified as a polypeptide covalently bound to gelatinase[(6)]. NGAL is expressed in a variety of human tissues, including lung, liver and kidney, in various pathologic states.3–5 Human NGAL consists of a single disulphide-bridged polypeptide with a molecular weight of 25 kDa[(7)].
Serum NGAL is associated with main indices of iron status (serum ferritin and TSAT) in hemodialysis patients, indicating that serum NGAL could reflect iron metabolism in these patients [(8)].
The aim of the study was to evaluaterole of serum NGAL as biomarker of iron deficiency in CKD patients.

Patients and Methods

Research Design

This is a prospective, Case-Control, Comparative study and was conducted at nephrology outpatient clinic and Internal medicine department of Zagazig University hospitals (from September 2020 to March 2021).

Subjects

Seventy two participants were divided into two equal groups:

- ***Group I:*** *Included 36 participants with no chronic kidney disease "GFR ≥ 90 mL/min/1.73 m^2" as a control group.*
- ***Group II:*** *Included 36 chronic kidney disease patients "GFR < 60 mL/min/1.73 m^2" on conservative treatments (not on Dialysis)as a case group*

Participants in this study were selected according to the following criteria:

- ***Inclusion Criteria:***
  - Male and female aged > 18 years old.
  - Patients with CKD "GFR < 60 mL/min/1.73 m^2" on conservative treatments (not on Dialysis).
  - Participants with similar age and sex to cases with no CKD "GFR ≥ 90 mL/min/1.73 m2".
- ***Exclusion criteria:***
  - Previous treatment with immunosuppressive drugs.
  - Active inflammatory diseases.
  - Acute infections.
  - Chronic or Acute Liver diseases.
  - Any malignancy or history of treatment with chemotherapy or radiotherapy.

Methods

I. Medical History:
II. Clinical Examination:

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III. Laboratory Investigations:

The following laboratory investigations for all participants in the study:

- Liver function tests including S. AST, S.ALT, serum Albumin and Total Pl. Proteins.
- Kidney function tests including Bl. Urea and serum Creatinine
- Serum Iron,
- Total cholesterol and serum triglycerides
- Serum NGAL levels will be measured by an enzyme-linked immunosorbent assay (ELISA).

IV. Estimated Glomerular Filtration Rate "eGFR" Calculation:

eGFR was calculated for all participants in the study by using the MDRD equation of the National Kidney Foundation eGFR mobile application.

"***GFR (mL/min/1.73 m2) = 175 × (Scr)-1.154 × (Age)-0.203 × (0.742 if female) × (1.212 if African American)"***

STATISTICAL ANALYSIS

All data were collected, tabulated and statistically analyzed using SPSS 22.0 for windows (SPSS Inc., Chicago, IL, USA) &MedCalc 13 for windows (MedCalc Software bvba, Ostend, Belgium).Continuous Quantitative variables e.g. age were expressed as the mean ± SD & median (range), and categorical qualitative variables were expressed as an absolute frequencies "number" & relative frequencies (percentage). One way ANOVA test was used to compare more than two groups of normally distributed data. Categorical data were compared using the Chi-square ($\chi 2$) test.Area under Curve (AUROC) was also calculated, criteria to qualify for AUC were as follows: 0.90 – 1 = excellent, 0.80-0.90 = good, 0.70-0.80 = fair; 0.60-0.70 = poor; and 0.50-0.6 = fail. The optimal cutoff point was established at point of maximum accuracy. All tests were two sided. $p< 0.05$ was considered statistically significant (S), $p < 0.001$ was considered highly statistically significant (HS), and $p \geq 0.05$ was considered non statistically significant (NS).

Results:

- There was no statistical significant difference between the studied groups as regard age, gender and body mass index "BMI"**Table (1)**.
- There was a statistical significant difference between the studied groups as regard Blood urea, serum creatinine and estimated glomerular filtration rate "eGFR"**Table (2)**.
- There was a statistical significant difference between the studied groups as regard serum ALT, serum AST, serum albumin and total plasma protein**Table (3)**.
- **Table (4)and Figure (1)** show that there was a statistical significant difference between both groups of the study as regard serum Iron.
- **Table (5) and Figures (2,3,4)** show that Regarding lipid profile, our results showed that in comparison with the normal healthy control group, patients in CKD group were having significantly lower HDL level and significantly higher LDL and Triglycerides levels, while regarding total cholesterol level there was no statistical significant difference between the CKD group and the control group.
- **Table (6) and Figure (5)** show that That there was a statistical significant difference between all groups of the study as regard serum NGAL level
- The validity of NGAL in diagnosis of CKD was assessed and we found out that NGAL at cut-off ≤112 ng/ml has sensitivity of "88.9%" and specificity was 75% **Table (7)**.

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Table (1): Basic characteristics of the studied groups:

	CKD Group (N = 36)	Control Group (N = 36)	t-value	p-value
Age: - Mean ± SD - Range	**49.9 ± 7.2** **35 - 64**	**48.3 ± 6.6** **34 - 64**	**0.988**	**0.163** **NS**
BMI: - Mean ± SD - Range	**27.9 ± 2.3** **25 - 32**	**27.3 ± 2.3** **24 - 31**	**1.188**	**0.119** **NS**
Gender: - Male - Female	**17 (47.2%)** **19 (52.8%)**	**22 (61.1%)** **14 (38.9%)**	χ^2 **1.398**	**0.237** **NS**

Table (2): Distribution of kidney function parameters among the studied groups:

	CKD Group (N = 36)	Control Group (N = 36)	t-value	p-value
Bl. Urea: - Mean ± SD - Range	**84.9 ± 6.7** **75 - 97**	**17.7 ± 2.6** **14 - 24**	**54.79**	**<0.001** **HS**
S. Creatinine: - Mean ± SD - Range	**1.82 ± 0.3** **1.3 – 2.3**	**0.75 ± 0.13** **0.4 – 0.9**	**23.04**	**<0.001** **HS**
Estimated GFR: - Mean ± SD - Range	**45.17 ± 8.46** **35.29 – 60.93**	**110.5 ± 30.5** **93 - 120**	**-14.38**	**<0.001** **HS**

Table (3): Distribution of liver function parameters among the studied groups:

	CKD Group (N = 36)	Control Group (N = 36)	t-value	p-value
S. ALT: - **Mean ± SD** - **Range**	**17.8 ± 2.4** **16 - 24**	**19.1 ± 0.47** **16 - 26**	**-2.49**	**0.008**
S. AST: - **Mean ± SD** - **Range**	**19.9 ± 1.4** **13 - 26**	**21.18 ± 1.2** **18 - 28**	**-2.492**	**0.007**

T.Pl. Protein: - **Mean ± SD** - **Range**	**7. 2± 0.4** **6.5 – 7.9**	**7. 5± 0.5** **6.5 – 8.2**	**-2.603**	**0.005**
S. Albumin: - **Mean ± SD** - **Range**	**3. 8± 0.1** **3.6 – 4.1**	**4. 0± 0.3** **3.5 – 4.5**	**-4.053**	**<0.001**

Table (4): Comparison of all groups as regardIron parameters:

	CKD Group (N = 36)	Control Group (N = 36)	t-value	p-value
S. Iron: (µg/dl) - **Mean ± SD** - **Range**	**65.0± 9.47** **45 – 82**	**93.18 ± 17.87** **75 – 140**	**-21.06**	**<0.001**

SD: stander deviation

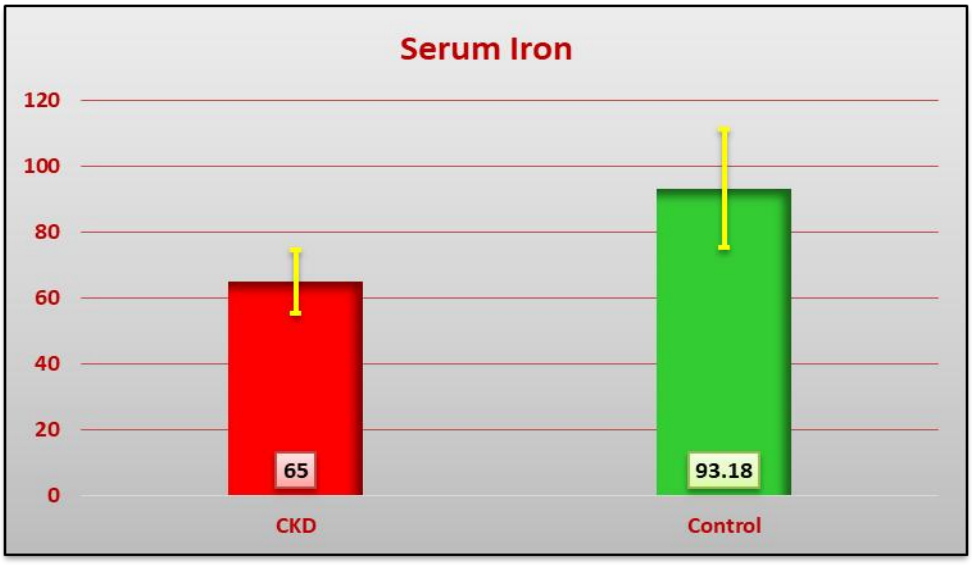

Figure (1): Serum iron distribution among studied groups.

Table (5): Distribution of Lipid profile parameters among the studied groups:

	CKD Group (N = 36)	Control Group (N = 36)	t-value	p-value
HDL: - **Mean ± SD** - **Range**	**47.93 ± 5.93** **36 – 57**	**57.90 ± 5.42** **47 – 70**	**-2.381**	**0.019**

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LDL: - Mean ± SD - Range	**129.5 ± 4.9** **121 – 135**	**119.9 ± 7.72** **100 – 129**	**2.586**	**0.009**
TG_3: - Mean ± SD - Range	**161.14 ± 95.09** **47.60 – 617.1**	**103.74 ± 11.95** **88 – 124**	**-2.490**	**0.013**
T. cholesterol: - Mean ± SD - Range	**206.42 ± 75.41** **76.9 – 397.5**	**174.86 ± 21.28** **125 – 198**	**-0.909**	**0.363**

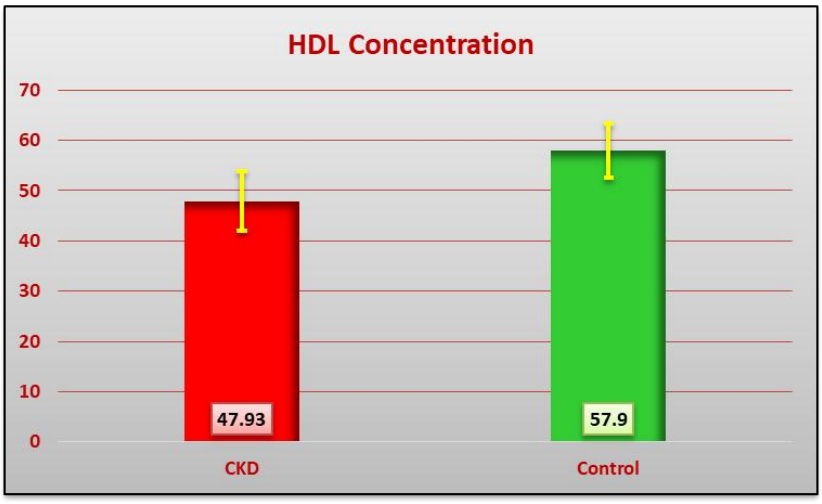

Figure (2): HDL distribution among studied groups.

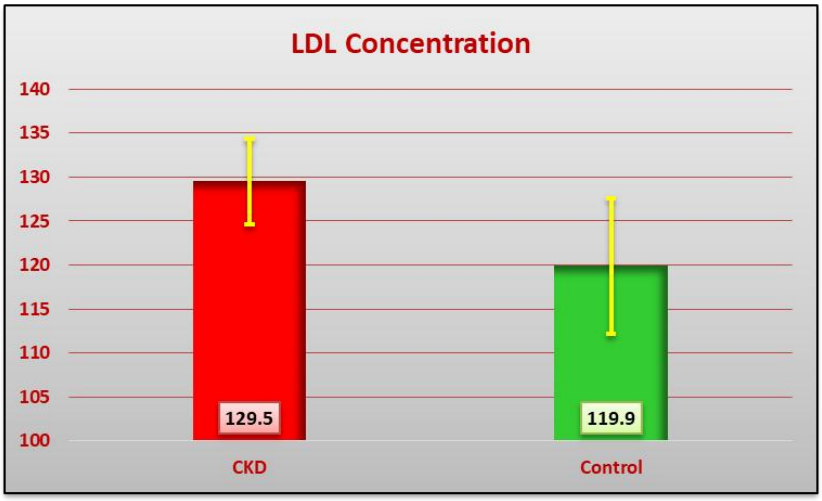

Figure (3): LDL distribution among studied groups.

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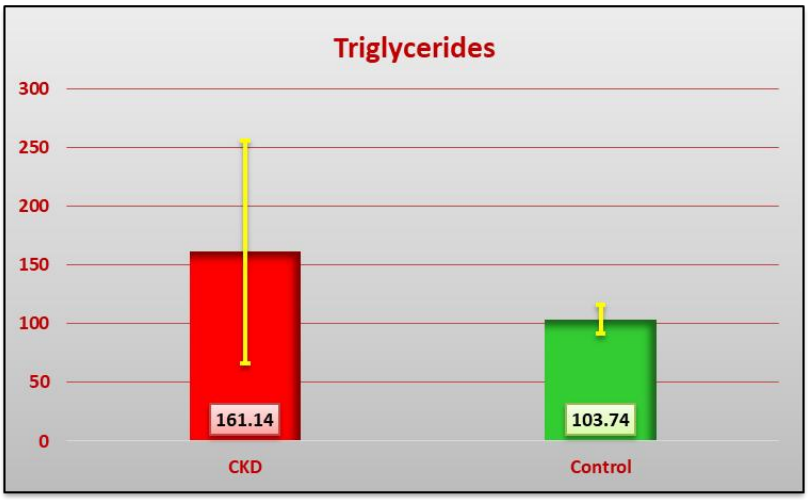

Figure (4): Triglycerides distribution among studied groups.

Table (6): Comparison of all groups as regard Serum NGAL

Variable	CKD Group (N = 36)	Control Group (N = 36)	t-value	P
NGAL: (ng/ml) Mean ± SD	288.4 ±46.92	107.9 ±43.7	17.007	<0.001

SD: stander deviation

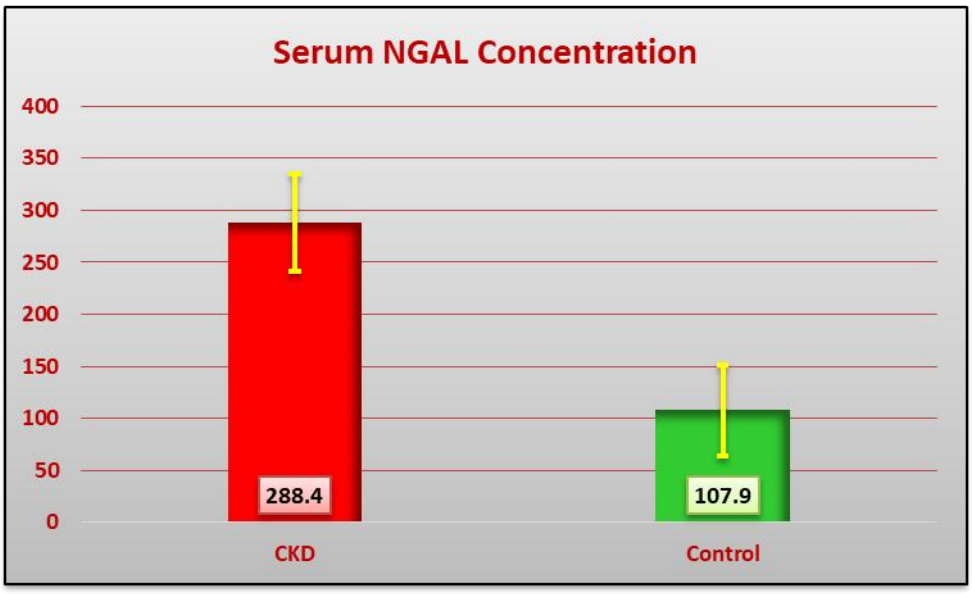

Figure (5): serum NGAL concentration distribution among studied groups.

Table (7): ROC Curve

Test Result Variable(S)	Area	StdError[a]	Asymptotic Sig[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
NGALng/ml	0.719	.089	0.025	0.544	0.894

Sensitivity and Specificity			
	Cutoff	**Sensitivity**	**Specificity**
NGALng/ml	**112**	**88.9%**	**75%**

Discussion

There was no significant difference between the two groups of the study as regard age, gender and body mass index (BMI) distributions with p-value: 0.163, 0.237 and 0.119 respectively as our study was designed on cross matched participant for age, gender and BMI in order to exclude their effect on serum NGAL level.

Bennett et al., [9] reported in their study that serum NGAL level was found to be associate with patients' ages and it increases with increase in patients' ages. Also, ***Bennett et al.,*** [10] and ***McWilliam et al.,*** [11] reported that NGAL was significantly higher in females than in males in both pediatric and adult populations.

Regarding kidney functions (Blood urea and serum creatinine) and eGFR distributions, our results showed that there was a statistical significant difference between the studied groups with p-value: < 0.001, these findings agree with that our studied groups constitute patients in different stages of CKD and non-CKD participants

Regarding liver functions tests (serum ALT, serum AST, total plasma protein and serum albumin), our results showed that serum ALT and serum AST were significantly lower in CKD group in comparison with those in the control group.

Our results were in agreement with ***Ray et al.,*** [12] who reported in their retrospective, hospital-based study on 100 patients that serum AST and ALT levels were significantly lower in CKD patients both without and with ESRD compared to controls. And concluded that levels of serum aminotransferases were low in CKD with and without ESRD and the levels become lower as the severity of CKD increases.

Also, were in agreement with ***Sette and Almeida Lopes,*** [13]who reported that the serum aminotransferase levels were lower in the patients with chronic kidney disease patients than in the patients with normal renal function.

The exact cause of low serum aminotransferase levels in CKD remains controversial, possible reasons include pyridoxine deficiency and/or the presence of an inhibitory substance in the uremic milieu [13].

Also, Total plasma protein and serum albumin were significantly lower in CKD group as compared to control group; these are in agreement with ***Rajagopalan et al.,*** [14]. This can be due to one or more of the following factors; loss of appetite, Diabetic gastroparesis, Mechanical compression of stomach and intestine in polycystic kidney disease, Immobility and reduced ability to purchase food, Inadequate dietary recommendations, Comorbidity, Socioeconomic factors (e.g. poverty, social deprivation), Depression, Chronic inflammation, Dialysis-associated amino acid and protein loss, Metabolic acidosis, Inadequate dialysis dose, Inadequate dental status, proteinuria and Complications of dialysis (e.g. nausea, hypotension) [15].

Serum iron were found to be higher in the control group than in the CKD group. these results are in agreement with ***Fishbane et al.,*** [16] who concluded that after The National Health and Nutritional Examination Survey (NHANES) data for NHANES III (1988 to 1994), It was found that low levels of iron tests, following National Kidney Foundation/Kidney Disease Outcomes Quality Initiative guidelines (either serum ferritin <100 ng/ml or TSAT < 20%) were present in most patients with reduced CrCl

Regarding lipid profile, our results showed that in comparison with the control group, patients CKD groups were having significantly lower HDL level and significantly higher

LDL and Triglycerides levels, while regarding total cholesterol level there was no statistical significant difference between the CKD group and the control group.
These findings were in agreement with ***Kawachi et al.,*** [17]***;*** *who* reported that Dyslipidemia is a common complication of CKD and lipoprotein metabolism alteration and is associated with the decline in GFR; hence, lipid profile depends on the level of kidney function and the degree of proteinuria.
Also, ***Attman et al.,*** [18]reported that Patients with CKD tend to have alterations in both HDL quantity and HDL quality. Even a mildly impaired GFR is associated with low HDL-C concentrations, which become progressively worse through ESRD.
The insignificant change in cholesterol level can be explained by that CKD without heavy proteinuria doesn't remarkably influence expressions of the gene of either hydroxyl-3-methylglutaryl-CoA reductase (HMG-CoA reductase) that is considered as the rate-limiting enzyme for cholesterol synthesis, or cholesterol 7ahydroxylase that is considered the rate-limiting enzyme for cholesterol catabolism and transformation to bile acids. Additionally, LDL receptor-dependent uptake of cholesterol acts a vital step during homeostasis of the cholesterol. Chronic kidney disease without significant glomerulosclerosis or heavy proteinuria doesn't change expression of the hepatic LDL-receptor gene [19].
Patients with CKD usually have hypertriglyceridemia due to an increased concentration of triglyceride-rich lipoproteins (VLDL, chylomicrons, and their remnants). Hypertriglyceridemia occurs because of both the delayed catabolism and the increased hepatic production of triglyceride-rich lipoproteins [20].
Regarding HDL, CKD patients have decreased levels of apolipoproteins AI and AII, the main components of HDL and the activity of lecithin–cholesterol acyltransferase, the enzyme important for the esterification of free cholesterol in HDL, is impaired and the activity of cholesterol ester transfer protein (CETP), which supports the transfer of cholesterol esters from HDL to triglyceride-rich lipoproteins, is increased [21].
Regarding serum NGAL level, our result showed that serum NGLA was higher in CKD group than in the non-CKD group with a statistical sginficance (p-value: <0.001).
In agreement with our findings was ***Kim et al.,*** [8]who reported in their study on 419 patients who had anemia. That plasma NGAL was found to be higher in CKD patients groups than in non-CKD groups.
Çiçek et al., [22]found in their study on 163 CKD patients including transplant patients and 82 healthy volunteers, that Serum hepcidin, Prohepcidin, NGAL, hypersensitive C-reactive protein and interleukin-6 levels were higher in patient groups compared to the control group.
In the study done by ***Xiang et al.,*** [23]they reported that NGAL level wasfound to be higher in CKD with anemia than those without anemia.
Different studies demonstrate the involvement of NGAL in adaptive stage of CKD. It was hypothesized that in CKD, the change in the physiology of this protein is comparable to the acute injury. Chronic injured kidney tubules produce enhanced NGAL. In conclusion, increased NGAL level is not only a result of decreased clearance, but also increased production [24].
This study showed that, NGAL is a good biomarker in diagnosis of iron status in CKD
In the study done by ***Kim et al.,*** [8]who they reported that the best cut-off value for plasma NGAL was ≤394 ng/ml with an associated sensitivity of 84.2% and specificity of 50.0%.

Conclusion:

Human neutrophil gelatinase-associated lipocalin is significantly higher when compared with controls and this means that it has an important rule iron metabolism in those patients. Serum NGAL can be a good biomarker for iron status in CKD patients.

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