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Serum Neutrophil Gelatinase-Associated Lipocalin and Iron Status in Predialysis Chronic Kidney Disease at Zagazig University

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ABSTRACT

Background: The burden of chronic kidney disease (CKD) is substantial. Anemia is a common feature of CKD.

Objective: The aim was to predict about iron status by serum neutrophil gelatinase-associated lipocalin (NGAL) levels and role of serum NGAL as biomarker of iron deficiency in CKD patients.

Patients and Methods: A prospective, case-control comparative study that was conducted at Nephrology Outpatient Clinic and Internal Medicine Department, Zagazig University Hospitals. Seventy two participants were divided into two groups: Group I included 36 participants with no chronic kidney disease and group II that included 36 chronic kidney disease patients. Laboratory investigations included iron parameters and serum NGAL levels were measured

Results: S. Iron, TIBC, S. Ferritin and calculated T. sat" were statistically analyzed and showed that there was a statistical significant difference between the two studied groups. NGAL level was higher in CKD group than in the non-CKD group. A significant positive correlation between serum NGAL and T sat & serum ferritin and there was a significant negative correlation between NGAL and eGFR, HB and serum iron.

Conclusion: Patients had significantly higher NGAL levels when compared to controls and this means that it had an important role in iron metabolism in those patients. There was statistically significant direct correlation between serum NGAL levels and serum ferritin levels and T. sat and inverse correlation with iron. Serum NGAL could be a good biomarker for iron status in CKD patients but not better than the ordinary used methods "serum ferritin and T. sat".

Keywords: Neutrophil Gelatinase, Associated Lipocalin, Iron status, Predialysis, CKD.

INTRODUCTION

Chronic kidney disease (CKD) is a global health burden affecting a large proportion of the population worldwide [(1)]. The burden of CKD is substantial. According to WHO global health estimates 864,226 deaths (or 1.5% of deaths worldwide) were attributed to this condition in 2012 [(2)].

The Kidney Disease Improving Global Outcomes (KDIGO) organization classified CKD by the degree of renal dysfunction, as measured by the estimated glomerular filtration rate (eGFR) and by the presence or absence of structural kidney abnormality or by other evidence of chronic kidney damage, particularly albuminuria. This classification gives five levels of dysfunction defined by eGFR (G1–G5) and three by albuminuria (A1–A3) [(3)].

Only a small proportion of people with CKD progress to end-stage kidney disease (ESRD) and renal replacement therapy (dialysis or transplantation), which represents major costs for health care systems and burden for patients [(3)].

ESRD is included under stage 5 of the KDIGO classification of CKD, where it refers to individuals with an estimated glomerular filtration rate less than 15 mL/min/1.73 m^2 or those requiring dialysis [(4)].

Anemia in CKD & ESRD is mostly due to erythropoietin (EPO) deficiency, inhibition of erythropoiesis by uremic solutes and reduction in red blood cell life span. Other causes include iron, B12 or folic acid deficiency or blood loss. Most patients with CKD can be effectively treated with erythropoiesis stimulating agents (ESA) [(5)].

Iron deficiency is a frequent cause of EPO resistance. An accurate assessment of iron status is important because both anemia and overtreatment with erythropoiesis stimulating agents (ESA) are associated with poor clinical outcomes [(6)].

Human NGAL was originally isolated from the supernatant of activated neutrophils and identified as a polypeptide covalently bound to gelatinase. [(7)]. NGAL can primarily bind siderophores, small hydrophobic molecules containing iron, transporting them into the cells to activate cytoplasmic iron-dependent pathways in order to protect the same cell from oxidative stress [(8)].

It is produced by injured nephron epithelia. 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 [(9)]. So, NGAL is considered as a novel biomarker of acute kidney injury (AKI) and a predictor of the progression of CKD [(10)]. The aim of the study was to predict about iron status by serum NGAL levels and role of serum NGAL as biomarker of iron deficiency in CKD patients.

PATIENTS AND METHODS

This is a prospective case-control comparative study and was conducted at Nephrology Outpatient

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Clinic and Internal Medicine Department, Zagazig University Hospitals from September 2020 to March 2021.

Ethical approval:
The study was approved by the Ethics Board of Zagzig University and an informed written consent was taken from each participant in the study.

Seventy two participants including both males and females were included in this study and 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 and **group II** that included 36 chronic kidney disease patients "GFR < 60 mL/min/1.73 m^2" on conservative treatments (not on Dialysis) as a case group.

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.

All patients were subjected to:
Medical History: Full detailed medical history was taken including the following points:
Clinical Examination: Clinical examination was done to exclude any hidden medical problems especially undiscovered DM, HTN or chronic liver diseases.
Laboratory Investigations: The following laboratory investigations were done for all participants in the study:

- Complete Blood Count (HB%, RBCs count, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration).
- Iron Parameters (serum iron, total iron binding capacity, serum ferritin and transferrin saturation was calculated).
- Serum NGAL levels will be measured by an enzyme-linked immunosorbent assay (ELISA).

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)"**

Abdominal Ultrasonography was done to all participants and no abnormal findings were found in the control group, while in CKD patients, there were changes in site, size, echogenicity and cortico medullary differentiation.

Statistical analysis

All data were collected, tabulated and statistically analyzed using SPSS 22.0. 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. Spearman's coefficient was calculated to assess relationship between NGAL and study parameters, (+) sign indicate direct correlation & (-) sign indicate inverse correlation, also values near to 1 indicate strong correlation & values near 0 indicate weak correlation. Area under curve (AUROC) was also calculated, all tests were two sided. $P \leq 0.05$ was considered statistically significant.

RESULTS

There was no statistical significant difference between the studied groups as regards age and gender (Table 1).

There was a statistical significant difference between both groups of the study as regards hemoglobin "Hb" level, red blood cells count "RBCs", packed cell volume "PCV", and mean corpuscular hemoglobin concentration "MCHC". While there was no significant difference between them as regard mean corpuscle volume "MCV" and mean corpuscular hemoglobin "MCH". There was a statistical significant difference between both groups as regards serum Ferritin, total iron binding capacity "TIBC" and serum iron and transferrin saturation (Tf Sat). There was a statistical significant difference between both groups as regards serum NGAL level (Table 2).

Table (3) showed that within the CKD group, there was a significant positive correlation between serum NGAL and Tf sat and serum ferritin and there was a significant negative correlation between NGAL and eGFR, HB and serum iron. There was no other significant correlation between NGAL and other variables**.**

The validity of NGAL in diagnosis of iron deficiency and in assessment of iron status in the CKD was assessed and we found out that NGAL at cut-off ≤ 112 ng/ml has the same sensitivity of serum ferritin "88.9%" and both have lesser sensitivity than transferrin saturation "94.4%". While NGAL specificity was 75% in comparison with serum ferritin "61.1%" and T. sat "100.0%". From these reading we considered that NGAL is a good biomarker for iron status in CKD patients but not better than serum ferritin and Tf. Sat (Table 4).

Table (1): Basic characteristics of the studied groups

	CKD Group (N = 26)	Control Group (N = 26)	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**
Gender: - Male - Female	**17 (47.2%)** **19 (52.8%)**	**22 (61.1%)** **14 (38.9%)**	**X^2** **1.398**	**0.237** **NS**

Table (2): Comparison of all groups as regard complete blood count, Iron parameters and serum NGAL.

	CKD Group (N = 26)	Control Group (N = 26)	t-value	p-value
	Mean ± SD	Mean ± SD		
Hemoglobin: (mg/dl)	**9.7 ± 2.2**	**14.5 ± 1.1**	**-19.06**	**<0.001**
RBCs: cell/cm^3	**3.9 ± 0.4**	**5.1 ± 0.3**	**-12.94**	**<0.001**
PCV: %	**33.1 ± 3.7**	**44.0 ± 3.3**	**-13.005**	**<0.001**
MCV: fL	**84.7 ± 3.5**	**86.0 ± 4.4**	**-1.345**	**0.091**
MCH: pg/cell	**28.5 ± 0.9**	**28.1 ± 1.0**	**1.618**	**0.055**
MCHC: g/dl	**33.6 ± 1.0**	**32.9 ± 0.9**	**3.486**	**<0.001**
S. Iron: (µg/dl)	**65.0 ± 9.47**	**93.18 ± 17.87**	**-21.06**	**<0.001**
TIBC: (µg/l)	**290.95 ± 21.52**	**349.54 ± 51.55**	**-19.75**	**<0.001**
Tf.Sat: (%)	**22.50 ± 4.25**	**27.05 ± 6.43**	**-9.91**	**<0.001**
S. Ferritin: (ng/ml)	**141.86 ± 7.55**	**94.54 ± 3.82**	**10.06**	**<0.001**
NGAL: (ng/ml)	**288.4 ± 46.92**	**107.9 ± 4.7**	**17.007**	**<0.001**

Table (3): Correlation of Serum NGAL and different parameters in CKD group

Varible	NGAL			
Age	**r-value**	**0.155**	**p-value**	**0.366**
BMI	**r-value**	**-0.045**	**p-value**	**0.792**
AST	**r-value**	**0.064**	**p-value**	**0.711**
ALT	**r-value**	**0.064**	**p-value**	**0.711**
ALB	**r-value**	**-0.056**	**p-value**	**0.748**
T.protein	**r-value**	**-0.222**	**p-value**	**0.194**
Urea	**r-value**	**0.109**	**p-value**	**0.526**
Cr	**r-value**	**0.167**	**p-value**	**0.329**
eGFR	**r-value**	**-0.933**	**p-value**	**0.000**
Ca	**r-value**	**0.156**	**p-value**	**0.364**
Ph	**r-value**	**0.192**	**p-value**	**0.262**
HB	**r-value**	**-0.544**	**p-value**	**0.001**
RBCs	**r-value**	**-0.219**	**p-value**	**0.199**
PCV	**r-value**	**-0.170**	**p-value**	**0.321**
MCV	**r-value**	**0.173**	**p-value**	**0.312**
MCH	**r-value**	**0.184**	**p-value**	**0.283**
MCHC	**r-value**	**-0.104**	**p-value**	**0.547**
Iron	**r-value**	**-0.974**	**p-value**	**0.000**
TIBC	**r-value**	**0.159**	**p-value**	**0.480**
Tsat	**r-value**	**0.935**	**p-value**	**0.00**
ferritin	**r-value**	**0.976**	**p-value**	**0.000**

Table (4): ROC Curve

Test Result Variable(S)	Area	Std Error	Asymptotic Sig	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
Ferritin ng/ml	**0.728**	**0. 092**	**0.019**	**0.548**	**0.909**
T Sat %	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
NGAL ng/ml	**0.719**	**0.089**	**0.025**	**0.544**	**0.894**
Sensitivity and Specificity					
	Cutoff		**Sensitivity**		**Specificity**
Ferritin ng/ml	**Standard**		**88.9%**		**61.1%**
T Sat %	**Standard**		**94.4%**		**100.0%**
NGAL ng/ml	**112**		**88.9%**		**75%**

DISCUSSION

Our results showed that patients in the CKD group had significantly lower Hb% in comparison with those in the control group. Furthermore, hematological investigations of RBCs count, PCV, MCV, MCH and MCHC" were done to all participants in this study to determine their anemic status. The statistical analysis of the collected data showed that there was a statistical significant difference between the two groups as regards RBCs count, PCV and MCHC. This is in agreement with **McClellan *et al.*** [(11)] who reported that anemia was present in 47.7% of 5222 predialysis patients with chronic kidney disease and Prevalence of anemia was strongly associated with declining glomerular filtration rate. Percentage of patients with hemoglobin ≤ 12 g/dL increased from 26.7% to 75.5% when glomerular filtration rate decreased from ≥ 60 mL/min/1.73 m^2 to < 15 mL/min/1.73 m^2. Prevalence of hemoglobin ≤ 10 g/dL increased substantially from 5.2% to 27.2% when glomerular filtration rate diminished from ≥ 60 mL/min/1.73 m^2 to < 15 mL/min/1.73 m^2."

Serum iron was found to be higher in the control group than in the CKD group. these results are in agreement with **Fishbane *et al.*** [(12)] who concluded that after the National Health and Nutritional Examination Survey (NHANES) data for NHANES III (1988 to 1994), they 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.

There was a significant difference between CKD group in comparison with the control group as regards TIBC. This result goes in agreement with **Malyszko *et al.*** [(13)] who reported that in CKD patients that TIBC was highest in control group while it was lower in CKD group.

Tf.sat was found to be statistically higher in control non-CKD group than in the CKD group. These results also goes in agreement with the previously mentioned study of **Malyszko *et al.*** [(13)] who reported that Tf.sat significantly is higher in control group than in CKD patients.

Serum ferritin level was significantly higher in CKD group than in the control group. This result goes in harmony with **Ali *et al.*** [(14)] who reported in their study that serum ferritin was significantly higher ($p < 0.001$) in CKD patients compared to control subjects.

Regarding iron parameters in CKD patients our results are in agreement with **Thang *et al.*** [(15)] who reported in their study on 175 CKD patients in order to evaluate serum iron and ferritin concentrations that mean serum iron and ferritin levels and TIBC in patients with CKD were significantly different from the values for the control group. In particular, the ferritin level was significantly higher in the study group (259 ng/mL) than in the control group (160 ng/mL). TIBC was lower in the study group (50.4 µmol/L) than in the control group (66.0 µmol/L). This is explained by that in patients with CKD anemia is a common comorbidity and contribute to many factors as CKD progresses, including impaired production of erythropoietin [(16)], and hepcidin-mediated iron restriction [(17)]. Also, iron deficiency plays an important role in pathogenesis of anemia in CKD patients [(18)]. TIBC findings in our study can be explained by that TIBC is low in anemia of chronic disease because there is excess iron, but it is not easily available. While, in iron-deficiency anemia, the TIBC is being elevated (higher than 400–450 mcg/dL) because iron stores are low [(19)].

The elevated serum ferritin can be explained to be due to the nonspecific protein synthesis compensating for protein loss in advanced CKD as well as the progression of inflammation in CKD patients [(15)]. Also, the CKD patients participating in this study were on iron therapy. Iron therapy was found to cause increase in serum iron, TIBC, T. sat and serum ferritin in CKD patients receiving iron than those how are not receiving iron, as reported by **Jairam, *et al.*** [(20)].

serum calcium level was significantly lower while serum phosphorus level was significantly higher in CKD groups as compared to control group. These findings are in agreement with **Mondry *et al.*** [21] where CRP was found to be significantly higher in CKD group in comparison with the control group. These are in agreement with **Sedighi and Abediankenari** [22] as they found that CRP was significantly higher in CKD patients compared to healthy populations,

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 (p-value: <0.001). **Çiçek *et al.*** [23] 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 patients groups compared to the control group. 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].

Regarding correlation between serum NGAL levels and other variables, our results showed that there was a significant positive correlation between serum NGAL and Tf sat & serum ferritin. While, there was a significant negative correlation between NGAL and eGFR, HB and serum iron. There was no other significant correlation between NGAL and other variables. Similar findings were reported by **Kim *et al.*** [10] who found that in pre-dialysis CKD group, univariate analysis showed plasma NGAL correlated positively with Tf sat (r = 0.452, P\0.001), serum ferritin (r = 0.190, P = 0.001), and hsCRP (r = 0.172, P = 0.003), and correlated negatively with eGFR (r = -0.211, P\0.001). In addition, there were no significant correlations between plasma NGAL and the other variables.

The validity of NGAL in diagnosis of iron deficiency and in assessment of iron status in CKD patients was assessed and we found out that NGAL at cut-off ≤112 ng/ml has the same sensitivity of serum ferritin (88.9%) and both have lesser sensitivity than transferrin saturation (94.4%). While NGAL specificity was 75% in comparison with serum ferritin (61.1%) and T. sat (100.0%). From these reading we could consider that NGAL is a good biomarker for iron status in CKD patients but not better than serum ferritin and Tf sat. In the study done by **Kim *et al.*** [10] 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%. The best cut-off value for serum ferritin was ≤ 181 ng/ml with an associated sensitivity of 50.9% and specificity of 66.7%. In the study done by **Xiang *et al.*** [9] they reported that the optimal NGAL cutoff value able to identify iron deficiency was found to be > 244.8 ng/mL, with a 73.01% sensitivity and 68.29% specificity.

CONCLUSION

Patients had significantly higher NGAL levels when compared to controls and this means that it has an important role in iron metabolism in those patients. There was statistically significant direct correlation between serum NGAL levels and serum ferritin levels, T. sat and inverse correlation with iron. Serum NGAL can be a good biomarker for iron status in CKD patients but not better than the ordinary used methods (serum ferritin and T. sat).

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