ORIGINAL ARTICLE

# Association between neutrophil geletinase-associated lipocalin and iron deficiency anemia in children on chronic dialysis

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**Background:** Iron deficiency anemia (IDA) in children with chronic kidney disease (CKD) is common and associated with higher risk of death. Neutrophil geletinase-associated lipocalin (NGAL) is a small 25 kDa glycoprotein, a member of lipocalin superfamily that released at the response of cellular stress from different cells. In addition, NGAL was studied as an iron regulatory glycoprotein and regulator of iron related gene. The aim of the current study was to determine any association between serum NGAL and body iron status markers in children on chronic dialysis. **Materials and Methods:** This correlation study was carried out between May 2012 and May 2013 and evaluated all dialysis patients less than 19 years in pediatric dialysis centers in Isfahan that didn't have exclusion criteria. They were 40 children, including 23 persons on hemodialysis (HD) and 17 persons dialyzed by peritoneal dialysis (PD). Furthermore, we selected 40 children as healthy controls. We examined the relationship between plasma NGAL levels and indices of anemia such as ferritin, transferrin saturation (TSAT) and serum iron (SI) in dialysis children. **Results:** Serum NGAL level in children on chronic dialysis (group including both PD and HD patients) was significantly higher than healthy controls ($P = 0.008$). Furthermore, in this group Serum NGAL level had inverse correlation with TSAT ($P = 0.04$, $r = -0.22$), SI ($P = 0.04$, $r = -0.2$), white blood cells ($P = 0.045$, $r = -0.26$) and serum ferritin ($P = 0.006$, $r = -0.3$). In addition, HD patients had higher serum NGAL level than PD patients ($P = 0.048$). **Conclusion:** High serum NGAL level in low TSAT group demonstrated that NGAL probably has an important role in IDA in children on chronic dialysis; therefore, it can be a new marker for diagnosis of IDA in CKD.

**Key words:** Hemodialysis, iron deficiency anemia, neutrophil gelatinise-associated lipocalin, peritoneal dialysis

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## INTRODUCTION

About 90 % of patients with end stage renal disease (ESRD) have anemia[1] and it is associated with higher risk of death.[2] It seems common cause of anemia in children with ESRD is erythropoietin (EPO) deficiency,[3] but in many situations it is hyporesponsive to EPO. Its causes are different such as iron deficiency anemia (IDA)[4] and inflammation.[5] Children on chronic dialysis often have IDA, because of multiple blood sampling, gastrointestinal bleeding, dietary restriction, and decreased intestinal absorption due to phosphate medication.[6] According to Kidney Disease Outcome Quality Initiative suggestion, transferrin saturation (TSAT) and ferritin are the first tools for evaluation of iron status in renal disease. TSAT <20, ferritin <200 are two cut-off points for IDA in patients on chronic dialysis.[7] There are some difficulties about these markers, first ferritin is an acute phase protein and is different in both sexes,[8] second TSAT also influences by malnutrition, chronic disease and inflammation, but it is more reliable than ferritin for evaluation of body iron status in patients on chronic dialysis.[9]

Neutrophil geletinase-associated lipocalin (NGAL) is a small 25 kDa glycoprotein, a member of lipocalin superfamily that released at the response of cellular stress such as inflammation and ischemia from different cells such as renal tubules, liver hepatocytes, endothelial, and smooth muscle cells.[10-16]

Now-a-days, serum NGAL uses as an acute kidney injury marker, but basically it is a bacteriostatic substance that released from secondary granules of neutrophils.[17,18] In addition, NGAL was studied as an iron regulatory glycoprotein and regulator of iron related gene.[19-23] Later investigations show NGAL causes anemia due to oxidative stresses. In experimental models, medullary and systemic NGAL causes erythropoiesis inhibition through induction of apoptosis and inhibition erythroid precursor

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differentiation.[24-28] NGAL also at the response of renal tubular cell injury, redirect systemic iron to proximal tubule cells of kidney.[21,29]

Another study was done former on adults on chronic hemodialysis (HD) that show relationship between IDA and serum NGAL.[30] However, on the basis of our knowledge, no study has yet performed about association between serum NGAL level and IDA in children on chronic dialysis.

The aim of current pilot study was to evaluate the association between serum NGAL and body iron status in children on chronic dialysis.

## MATERIALS AND METHODS

### Study design and participants

This correlation study was performed between May 2012 and May 2013. We selected for current study children who received treatment for ESRD (either HD or peritoneal dialysis [PD]) for a minimum of 6 months and didn't have exclusion criteria at all Isfahan dialysis centers.They were 40 patients, including 23 persons on HD and 17 person dialyzed by PD. Exclusion criteria were the presence or a recent history of bleeding, malignancy, liver, thyroid or infectious disease, recent increase in leukocyte count or treatment with immunosuppressor drugs.

HD group were on regular treatment for dialysis, 3 times a week about 4 h/each session on standard bicarbonate dialysis. HD and PD patients matched for age and sex and duration of dialysis. All dialysis children were on recombinant EPO treatment for at least 6 mount, none of them received iron or RBC in past 2 mounts. They were normotensive without edema. Causes of ESRD in these children were hypoplasia/dysplasia ($n$ = 22), reflux nephropathy ($n$ = 8), drug reaction ($n$ = 2), metabolic disease ($n$ = 3), and unknown ($n$ = 5).

Control group was selected of healthy children that referred to clinic for assessment of body iron status. This group was matched for age, sex with dialysis children. They were on regular diet without consumption supplementary iron drugs.

### Procedures and variable assessment

Sampling was carried out at hospital dialysis unit for HD patients and at routine laboratory assessment for PD patients. Samples transferred on ice to laboratory unit and quickly freeze on –20°C. Fasting serum samples were obtained in the early morning for biochemical studies. These biochemical variables were Cr, urea, hemoglobin, hematocrit, leukocyte count, serum iron (SI), TSAT, ferritin, transferrin iron binding capacity (TIBC), albumin, alkaline phosphates, para thyroid hormone, high-sensitivity C-reactive protein (hsCRP), and NGAL. All biochemical blood samples were collected before mid-week dialysis session in HD group.

Markers that measured for assessment of body iron status were SI, TSAT, ferritin, TIBC.

SI, ferritin measured by radioimmunoassay.TIBC measured through spectrophotometry. TSAT calculated by this formula: $\frac{SI}{TIBC} \times 100$. Serum CRP was obtained to demonstrate the presence of inflammation and measured by immunoturbidometry. Plasma NGAL level measured by researched enzyme-linked immunosorbent assay (LOT. NO. KIT EKO 853, BOSTER biological Technology Co., Ltd.; Wuhan, China). Minimum detection limit was 10 ng/mL and coefficient of variation was <5%.

Other measurements were performed by routine automated laboratory tests.

Iron deficiency was defined as TSAT <20% and used for subdivision of patients to two groups to assessment correlation between serum NGAL level and iron status in children on chronic dialysis.

### Statistics analysis

Data presented as mean ± standard deviation. Data analysis was performed by SPSS version 16 (SPSS Inc., Chicago, IL, USA). Normality of variables was assessed by Kolmogorov–Smirnov test.

Differences between groups were determined by unpaired $t$-test and level of significance was 0.05. Pearson correlation coefficient test was used to show relationship between serum NGAL and different data. Chi-square test used for nonnumerical variables.

## RESULTS

Table 1 illustrates the main characteristics of this study In group, including both HD and PD patients the mean age was 9 ± 2. Mean serum NGAL level was 327.3 ± 29.8 ng/mL, which was significantly higher than healthy controls ($P$ = 0.008).

Hematologic markers (Hb and Hct) and iron status markers (TSAT, SI) in this group was significantly higher than healthy controls ($P$ < 0.001). Inflammatory markers (such as hsCRP and ferritin), urea and creatinine as expected were higher than healthy controls ($P$ < 0.05). Serum NGAL level in this group had inverse correlation with TSAT ($P$ = 0.04,

$r = -0.22$), SI ($P = 0.04$, $r = -0.2$), white blood cells ($P = 0.045$, $r = -0.26$), and serum ferritin ($P = 0.006$, $r = -0.3$). Serum NGAL level in HD group also had inverse correlation with TSAT ($P = 0.038$, $r = -0.18$), SI ($P = 0.042$, $r = -0.21$).

Correlation between serum NGAL level in PD group and iron status markers such as SI, TSAT, and ferritin was reverse, but not significant ($P = 0.063$, $r = -0.34$).

### Iron balance in children on chronic dialysis

Children on chronic dialysis was subdivided to two groups on the basis on absence or presence of IDA according to TSAT <20 (low TSAT group) or TSAT >20 (high TSAT group), so 17 patients (45%) belonged to low TSAT group and 23 patients (55%) belonged to high TSAT group, surprisingly between low TSAT group only 8 person (50%) simultaneously presented serum ferritin level under 200. Differences between low TSAT group and high TSAT group were summarized in Table 2. Serum NGAL in low TSAT group was significantly higher than high TSAT group ($P = 0.04$). SI ($P < 0.001$), TIBC ($P = 0.04$), TSAT ($P < 0.001$) were significantly higher in high TSAT group. Differences in Hb, Hct, and ferritin between these two groups were not significant. Creatinine and urea in high TSAT group was higher than low TSAT group, but not significant ($P > 0.05$).

### Comparison between hemodialysis and peritoneal dialysis patients

Table 3 summarized results of comparison between HD and PD patients.

A total of 17 patients (41%) belonged to PD group and 23 patients (49%) belonged to HD group. HD patients presented significantly higher TSAT ($P = 0.048$), SI ($P = 0.047$), NGAL (0.04), urea ($P = 0.010$), and ALP ($P = 0.001$) than PD patients. Differences between hemoglobin and hematocrit between PD and HD patients were not significant ($P > 0.05$). Urea was significantly higher in PD than HD patients ($P = 0.010$). Creatinine was higher in PD patients, but not significant ($P = 0.34$).

## DISCUSSION

There are some points to discussion in our study.

First, serum NGAL level in children on chronic dialysis was higher than healthy controls. We expected this consequence because according to previous studies NGAL was known as an acute phase protein that released by injured tissue such as renal cells.[31] In renal disease serum NGAL known as a primary indicator of organ damage[32] and in patients with chronic kidney disease (CKD) increased serum and urine level of NGAL is correlated with residue of renal function.

**Table 1: Main clinical and laboratory characteristic of the patients**

| Parameter | Patients ($n = 40$) | Controls ($n = 40$) | *P* value |
|---|---|---|---|
| Gender (male/female) | 19/21 | 11/9 | 0.61 |
| Age (years) | 9±2 | 9±5 | 0.898 |
| Creatinine (mg/dL) | 6.21±0.3 | 0.75±0.2 | <0.001 |
| Urea (mg/dL) | 53.9±21 | 8.5±2 | <0.001 |
| Hemoglobin (g/dL) | 8.8±2.04 | 12.5±1.11 | <0.001 |
| Hematocrit (%) | 27.05±6.26 | 36.6±3.2 | <0.001 |
| ALP (IU/L) | 455.31±289 | – | – |
| PTH (pg/dL) | 141.4±156 | – | – |
| WBC ($n \times 10^6$) | 7.269±3.55 | 6.31±2.1 | 0.06 |
| Albumin (g/dL) | 5.44±12.43 | 5.34±10.2 | 0.44 |
| hsCRP (mg/L) | 6.2±17 | 0.11±0.03 | 0.04 |
| Serum iron (mcg/mL) | 75.05±28 | 105±12.2 | <0.001 |
| TIBC (mg/dL) | 225±83 | 402.1±53.2 | 0.01 |
| TSAT (%) | 26.07±12.6 | 34.8±6.69 | 0.006 |
| NGAL (ng/mL) | 327.38±29.8 | 142.7±78.7 | 0.008 |
| Ferritin (ng/mL) | 166.5±164.5 | 118.7±44.02 | 0.208 |

hsCRP = High-sensitivity C-reactive protein; ALP = Alkaline phosphatase; TSAT = Transferrin saturation; TIBC = Transferrin iron binding capacity; PTH = Para thyroid hormone; NGAL = Neutrophil geletinase-associated lipocalin; WBC = White blood cell

**Table 2: Comparison between low TSAT group and high TSAT group in main clinical**

| Parameters | TSAT <20% ($n = 16$) | TSAT >20% ($n = 24$) | *P* value |
|---|---|---|---|
| Gender (male/female) | 7/9 | 14/10 | 0.56 |
| Age (years) | 9±5.5 | 9.6±0.4 | 0.73 |
| Creatinin (mg/dL) | 6.49±2.6 | 0.04±0.44 | 0.564 |
| Urea (mg/dL) | 55.8±15 | 52.62±23 | 0.658 |
| Hb (g/dL) | 9.25±1.9 | 8.6±2.1 | 0.35 |
| Hematocrit (%) | 28.57±5.4 | 26.03±6.6 | 0.21 |
| ALP (IU/L) | 424.15±244 | 522.8±387 | 0.505 |
| PTH | 113.7±69 | 159.9±194 | 0.36 |
| WBC ($n \times 10^6$) | 7.51±3.69 | 7.10±3.53 | 0.72 |
| Albumin (g/dL) | 3.48±0.78 | 6.74±16 | 0.424 |
| hsCRP (mg/dL) | 10.36±23 | 3.43±10.2 | 0.214 |
| Serum iron (mcg/mL) | 54±15 | 88.7±27 | <0.0 01 |
| TIBC (mg/dl) | 249.6±67 | 209.2±89 | 0.04 |
| TSAT (%) | 13.8±4.32 | 34.25±9.19 | <0.001 |
| Serum ferritin (ng/mL) | 205.06±186 | 140.9±146 | 0.232 |
| NGAL | 371.3±314 | 261.4±267 | 0.04 |

hsCRP = High-sensitivity C-reactive protein; ALP = Alkaline phosphatase; TSAT = Transferrin saturation; TIBC = Transferrin iron binding capacity; PTH = Para thyroid hormone; NGAL = Neutrophil geletinase-associated lipocalin; WBC = White blood cell

Second, there was a significant reverse correlation between serum NGAL and SI, TSAT, and ferritin in our study, this consequence can illustrated on the basis of previous investigations such as in one study, NGAL level was measured in blood, liver, and spleen after experimental induction of different types of anemia such as blood loss (phlebotomy, anemia induced by bleeding), IDA (sideropenic anemia) and hemolytic anemia (after phenyl

**Table 3: Comparison between HD and PD patients in main parameters**

| Parameters | PD (*n* = 17) | HD (*n* = 23) | *P* value |
|---|---|---|---|
| Gender (male/female) | 7/10 | 12/11 | 0.246 |
| Age (years) | 8±1 | 10±1 | 0.268 |
| Hemoglobin (g/dL) | 9.26±0.96 | 8.7±4.5 | 0.403 |
| WBC ($n\times10^6$) | 8364±890 | 4622±700 | 0.09 |
| Serum ferritin (ng/mL) | 199±43 | 145±32 | 0.316 |
| hsCRP (mg/dL) | 8±6 | 2.5±1.9 | 0.349 |
| Serum iron (mcg/Dl) | 66±8 | 83±4.4 | 0.047 |
| TIBC (mg/dL) | 271±8 | 185±14 | 0.001 |
| Creatinine (mg/dL) | 6.6±0.7 | 5.6±0.35 | 0.345 |
| Urea (mg/dL) | 63±6 | 45±0.6 | 0.010 |
| ALP (IU/L) | 414±53 | 463±69 | 0.001 |
| Albumin (g/dL) | 3.3±0.18 | 7.15±3.56 | 0.361 |
| Hematocrit (%) | 28.7±1.36 | 26±1.38 | 0.191 |
| PTH (pg/m) | 119.7±16 | 158±43 | 0.456 |
| NGAL (mg/mL) | 234.752±639 | 368.386±612 | 0.04 |
| TSAT (%) | 19.2±5.24 | 35.01±8.21 | 0.048 |

PD = Peritoneal dialysis; HD = Hemodialysis; hsCRP = High-sensitivity C-reactive protein; ALP = Alkaline phosphatase; TSAT = Transferrin saturation; TIBC = Transferrin iron binding capacity; PTH = Para thyroid hormone; NGAL = Neutrophil geletinase-associated lipocalin; WBC = White blood cell

hydrazine injection). In three upper situations, NGAL significantly increased in multiple organs such as liver and in two former conditions SI was decreased.[31] In one animal model, NGAL was able to separate iron from transferrin and redirect it from liver and spleen to renal proximal tubules to induce proliferation of these cells and inhibit their apoptosis.[30] In this study, serum NGAL level in low TSAT group was significantly higher than high TSAT group. According to findings of our study and previous investigations, we found out that NGAL except its role in prevention of erythropoiesis has an important role in production of IDA in CKD, it seem that mechanism of this effect is complicated, but its function in separating iron from transferrin and redirects it to proximal renal tubules can plays an important role.

In addition, comparison between low TSAT group and serum ferritin level under 200 revealed that ferritin was not an ideal marker for diagnosis IDA in CKD. Therefore, serum NGAL can be a new marker for diagnosis of IDA in CKD that more investigations with more participants need in future.

Third, it is well-known that HD patients have severe anemia than PD patients even with EPO treatment but SI in HD patients was higher than PD patients as a result of recurrent blood transfusion.[33] In addition to PD patients have better renal residual function than HD patients,[33] therefore on the basis of our findings in this study, we can explain these findings. NGAL level was higher in HD patients because of severe anemia and lower renal residual function in these patients.

### Limitations

There were some limitations in the current study. First, participants were not as high as can extend these findings to all children on chronic dialysis. Second, we didn't correct IDA and then measure NGAL level to identify cause-and-effect relationship between serum NGAL and IDA.

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## AUTHOR'S CONTRIBUTIONS

MY carried out the design and coordinates the study. ZN carried out the design and coordinates the study, and carried out all the experiments and prepared the manuscript. AB and AM provide assistance in the design of the study. NS and HM provided assistance for most experiments. All authors have read and approved the content of the manuscript.

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