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Research Article

Neutrophil Gelatinase-Associated Lipocalin (NGAL) as a Biomarker of Iron Deficiency in Hemodialysis Patients

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Abstract

Iron deficiency is often present in Hemodialysis (HD) patients. NGAL is a novel regulator of iron related gene that may contribute to anemia in HD patients. We conducted this study to evaluate the relationship of NGAL and the iron status in HD patients. Prospective cohort study was carried out, in between Dec 2012 to Mar 2014. The total number of the study was 120 hemodialysis patients among them 67 males and 53 females. According to transferrin saturation (TSAT) the eligible participants were divided into two equal subgroups. Group IA: TSAT <20% (iron deficiency). Group IB: TSAT ≥ 20% (without iron deficiency). Additional 60 healthy volunteers as control group (Group II). NGAL levels, indices of anemia, such as ferritin, TSAT and serum iron were measured in all subjects. NGAL level was significantly higher in HD patients than the healthy control group; furthermore, HD patients with TSAT < 20 had lower NGAL values than HD patients with TSAT ≥ 20. Surprisingly NGAL values significantly increased to comparable level after correction of iron deficiency by i.v. iron administration. The mean value of S. iron, S. transferrin, TSAT and S. ferritin were significantly higher in group IB than group IA. NGAL cutoff level of ≥461 ng/ml had a greater sensitivity and specificity than ferritin level of <200 ng/ml and TSAT level of < 20% in identifying iron deficiency among HD patients. All study parameters were correlated with NGAL level: Only SCr (P<0.05), ALP (P<0.01), HB (P<0.001), hsCRP (P=<0.001), S. Iron (P<0.001), TSAT (P<0.001) and ferritin (P<0.001) were positively correlated with NGAL level. The potential use of NGAL measurement may be valuable as a novel marker of iron status among hemodialysis patients.

Keywords: NGAL; Hemodialysis; Iron deficiency anemia; Iron status

Introduction

Anemia is defined as a reduction in one or more of the major red blood cell measurements; hemoglobin concentration, hematocrit, or red blood cell count. The World Health Organization defines anemia as a hemoglobin level less than 13 g/dL in men and post-menopausal women, and less than 12 g/dL in pre-menopausal women [1]. Anemia is a universal complication among patients with End Stage Renal Disease (ESRD), due mainly to impaired erythropoietin synthesis by the diseased kidneys, and an absolute or functional iron deficiency [2]. Other contributing factors include inflammation, regular blood loss, haemolysis, vitamin deficiency, hyperparathyroidism and medications [3].

The Kidney Disease Outcomes Quality Initiative (K/DOQI) anemia work groups suggest that serum ferritin and the TSAT should be considered primary tools in the assessment of iron status in nephropathic subjects; in particular, serum ferritin levels of <100 ng/mL in pre-dialysis chronic kidney disease (CKD) patients or<200 ng/mL in chronic HD patients, and/or TSAT values <20% (in both groups) should reflect an underlying condition of low iron deposits, thus being suggested as cut-off values for deciding upon opportune therapeutic strategies [4].

Serum ferritin was a reliable index of iron storage in HD patients. This protein is an acute-phase reactant, is markedly influenced by malnutrition and has important gender differences, thus making it a less than an ideal tool for identifying iron deficiency. For example, the presence of inflammation can explain the apparent paradoxical coexistence of high ferritin (>500 ng/mL) and low TSAT (<20%) levels frequently found in HD patients [5]. Although TSAT may also be influenced by concomitant conditions, such as reduced transferrin synthesis, inflammation or daily fluctuations, it appears to indicate iron stores in HD patients more reliably than serum ferritin [6].

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 [7-8]. NGAL was originally identified as a product of activated human neutrophils, capable of acting as a bacteriostatic agent by sequestering bacterial ferric siderophores and interfering with bacterial iron uptake [9].

NGAL is a novel iron-carrier protein [10] and seems to be one of the earliest biomarker of acute kidney injury [11], and also NGAL represents a novel predictive biomarker for CKD and its outcomes [12].

However, reports have implicated NGAL upregulation as a mechanism that contributes to anemia in the setting of chronic inflammation. In experimental models, systemic and medullary NGAL has been demonstrated to induce inhibition of erythropoiesis

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Table 1: Demographic, laboratory characteristics of the study.

Parameter	Group IA TSAT <20%	Group IB TSAT ≥ 20%	Group II Control group	F	P
Gender (M/F)	0.8/1	1.8/1	1.5/1	1.7	0.42 NS
Age (years)	51.3 ± 9.9	51.8 ± 8	53.7 ± 5.4	0.27	0.75 NS
Cr. mg/dl	9.6±1.4[c]	9.7±1.2[c]	0.94±0.2	23.7	<0.001*
Urea mg/dl	163.3±30.1[c]	182.4±22.3[c]	17.5±2.5	26.3	<0.001*
CaxP mg^2/dl^2	41.7±11.7[c]	40.4±8.3[c]	30.3±1	10.7	<0.001*
ALP IU/L	74.1±16.8	137.2±84.4[ac]	82.1±2.6	6.2	0.003*
Albumin mg/dl	3.5 ± 0.2[c]	3.7 ± 0.15[c]	4.3 ± 0.26	5.4	0.007*
hsCRP mg/l	23.8±17.6[bc]	10.9 ± 4.1[c]	0.3 ± 0.03	14.9	<0.001*
Uric acid mg/dl	6.3 ± 0.86[c]	5.8 ± 0.8[c]	4.8 ± 0.3	12.1	<0.001*
HB gm/dl	9.7 ± 0.6[c]	10.8 ± 1[c]	13.8 ± 0.46	95	< 0.001*
WBC 10^3	7.4 ± 0.3	6.8 ± 0.9	6.9 ± 1.3	1.28	0.28 NS
HCT%	32.5 ± 2.6[bc]	34.9 ± 3[c]	42.8 ± 2.1	50.9	< 0.001*
S. Iron mcg/ml	45 ± 7.7[bc]	89.9 ± 15	89.3 ± 3.9	89	< 0.001*
S. Transferrin mg/dl	172.6 ± 26[c]	223.4 ± 33[c]	301.6 ± 8	74.9	< 0.001*
TSAT %	13.9 ± 3.2[bc]	34.8 ± 8	27.7 ± 2.5	11.6	< 0.001*
S. ferritin ng/ml	189 ± 141.7[c]	450.6 ± 280[ac]	142.8 ± 13	70.99	< 0.001*
NGAL(ng/ml)	442 ± 46[c]	495.4 ± 55.9[ac]	40.1 ± 4.1	27.5	< 0.001*

[a]Indicate a significant difference as compared to Group IA
[b]Indicate a significant difference as compared to Group IB
[c]Indicate a significant difference as compared to Group II
* Significant. NS. None significant

through induction of apoptosis and arrest of differentiation of erythroid progenitor cells [13].

The aim of the present study is to assess the relationships of NGAL and iron balance, and its utility as a biomarker of iron deficiency in in HD patients

Patients and Methods

Study design and population

This prospective cohort study was carried out in hemodialysis unit of the Zagazig university hospital, in between Dec 2012 to Mar 2014; the aim of the study was explained to all participants and all of them gave informed consent to participate in this study in compliance with the local Institutional Ethics Committee and conformed to the Helsinki Declaration.

Eligible participants included patients on maintenance hemodialysis for at least 1 year. Participants were excluded from the study if suffered from the recent history of bleeding, malignancy, liver diseases, thyroid disorders, infectious diseases, alterations in leukocyte count and/or treatment with steroids or immunosuppressive medication.

Demographic information was collected, the total number of the study was 120 hemodialysis patients among them 67 males and 53 females (1.3:1), on maintenance hemodialysis three times weekly using 3.5 mEq/l dialysate calcium, duration of each dialysis was 4 hours, protocols were not changed during the study with adequate dialysis treatment (Kt/V>1.2).

All subjects had been dry-weight, stable for at least 2 months before the study was started and had achieved a normotensive edema-free state. All HD patients had been on recombinant erythropoietin therapy for at least 6 weeks and none had received intravenous iron administration or packed red cell transfusion in the 2 months preceding the start of the study. The main causes of ESRD were chronic glomerulonephritis 33 (27.5%), diabetic nephropathy24 (20%), interstitial nephropathy 18 (15%), unknown 17 (14%), hypertension 15 (12.5%) and obstructive uropathy 13 (11%).

Iron deficiency was defined as TSAT <20% [4]. Accordingly the eligible participants in this study were divided into two subgroups.

Group IA: TSAT <20% (iron deficiency), 60 subjects (27 males and 33females, their age ranged from 38 years to 68 years (mean 51.3 + 9.9 y) Group IB: TSAT ≥ 20% (without iron deficiency), 60 subjects (39 males and 21 females their age ranged from 40 years to 67 years (mean 51.8 + 8 y)

Group II: Control group was selected of healthy 60 subjects with comparable age, sex to HD patients, for assessment of body iron status. They were on a regular diet without consumption supplementary iron drugs

Follow up

Group IA: TSAT <20% (iron deficiency), were given IV iron infusion 200–500 mg during hemodialysis session, up to correction of iron deficiency (Corrected group IA)

Laboratory measurements

Peripheral venous blood samples were taken in the midweek interdialytic day. All patients were subjected laboratory analysis for complete blood picture (CBC) including haemoglobin (HB) (g/dl), haematocrit % (HCT), uric acid, fasting blood sugar, serum albumin,

Table 2: Iron parameters of HD patients in group IA (TSAT<20%) after correction of iron deficiency.

Variable	Group IA	Corrected Group IA	Paired t	P
Iron	45 ± 7.7	73.3 ± 8.9	72.7	<0.001
Transferrin	172.6 ± 26	215.1 ± 26.8	11.57	<0.05
TSAT	13.9 ± 3.2	23.1 ± 1.7	22.5	<0.001
Ferritin	189 ± 141.7	309.6 ± 187.9	26.7	<0.001
NGAL	442 ± 46	490 ± 50.1	46.5	<0.001

blood urea nitrogen (BUN), creatinine (SCr), serum alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), Ca, P , intact parathyroid hormone (i-PTH) and high-sensitivity C-reactive protein (hsCRP), according to standard methods used in the routine clinical laboratory. Iron balance was assessed by measuring the total serum iron, serum transferrin, serum ferritin and transferrin saturation (TSAT) calculated according to the following formula: (serum iron/serum transferrin) ×70. 9.

NGAL was measured in the blood using the Enzyme linked immunosorbent assay (ELISA) commercially available kit (BioVendor. Product NO. RD191102200R. Czech Republic), according to the manufacturer's instructions. All measurements were made blind, in triplicate. NGAL levels were expressed as ng/mL.

Statistical analysis

Data were collected, entered, analyzed and presented as means standard deviations, analysis of variance (ANOVA and LSD tests). The correlation between variables is calculated using the Pearson's and the Spearman correlation tests. Chi square (χ^2) test and the criterion for statistical significance were set at $P< 0.05$. All calculations were carried out using a standard statistical package (SPSS version19. Inc. in Chicago, USA).

Results

Demographic data and characteristic of study

NGAL level was significantly higher in HD patients than the healthy control group; furthermore, HD patients with TSAT < 20 (Group IA) had lower NGAL values than HD patients with TSAT ≥ 20 (Group IB). There were no significant differences in between the 3 groups regarding age, gender and WBC. Really the mean value of SCr, urea and Ca x P ratio were significantly higher in HD patients than the healthy control group. S albumin was significantly lower in HD patients than the healthy control group. hsCRP significantly higher in HD patients than the healthy control group, furthermore hsCRP was significantly higher in group IA than group IB. The mean value of HB and HCT were significantly lower in HD patients than the healthy control group, furthermore HB and HCT were significantly lower in group IA than group IB. Finally S. iron, S. transferrin, TSAT and S. ferritin were significantly higher in group IB than group IA. (Table 1)

Table 3: Sensitivity and specificity of Ferritin, NGAL and TSAT, using ROC analysis.

Variable	No of patients & %		Sensitivity	Specificity	PPV	NPV	Accuracy
1-Ferritin							
≥ 200 ng/ml	87	72.5%	72.5	100	100	47.6	78
< 200 ng/ml	33	27.5%					
2-NGAL							
≥ 461 ng/ml	90	75%	75	100	100	50	80
< 461 ng/ml	30	25%					
3-TSAT							
> 20%	60	50%	50	100	100	33.3	60
< 20%	60	50%					

NGAL levels and iron treatment

The mean value of serum iron, TAST, serum ferritin was significantly coming up after correction of iron deficiency, also NGAL values significantly increases to comparable level after correction of iron deficiency by i.v. iron administration (Table 2).

Correlates of NGAL and other parameters

All study parameters were correlated with NGAL level: Only SCr (r=0. 38;P=<0.05), ALP (r=0. 46;P<0.01), HB (r=0. 61;P<0.001), hsCRP (r=0. 64;P=<0.001), S iron (r=0. 73;P<0.001), TSAT (r=0. 85;P<0.001) and serum ferritin (r=0. 74; P<0.001) were positively correlated with NGAL level.

Sensitivity and specificity of Ferritin, NGAL and TSAT

The best NGAL cut-off value able to identify iron deficiency was found to be <461 ng/mL and had a greater sensitivity and specificity than S ferritin level of <200 ng/ml and TSAT level of < 20% in identifying iron deficiency among HD patients. Therefore NGAL showed a good diagnostic power in identifying iron deficiency among HD patients than S ferritin and TSAT Table 3.

Discussion

Diagnosis of iron deficiency anemia with currently available tests is rendered difficult in hemodialysis patients [14]. Consequently the impressiveness to assess the utility of NGAL as a novel marker of iron deficiency in in HD patients

In the current study, we observed that an increase of the circulating NGAL levels in hemodialysis patients than the control healthy subjects. NGAL basically had an innate anti-bacterial factor that released from secondary granules of neutrophils [9]. It has been shown that innate immune defense and neutrophil granulocytes functions were impaired in chronic kidney disease [15]. Despite of neutrophil dysfunction in CKD and non-significant difference between the three studied groups regarding WBC count, NGAL level was elevated in our hemodialysis patients. It is now widely accepted that NGAL is a true acute-phase factor that can be released by virtually almost every injured tissue, often becoming a marker of disease severity [7]. NGAL represents a novel predictive biomarker for CKD and its outcomes [13]. In addition, we observed that NGAL levels not only rise in CKD patients on HD, but also positively correlated with SCr. So we can speculate that NGAL level correlate with residual renal function. However the high level of NGAL in HD patients and its correlation with residual renal function was supported by many authors [16-18]

In addition, we observed in this study, serum NGAL level in HD patients with TSAT <20% group was significantly lower than HD patients with TSAT ≥20% group, astonishingly correction of iron deficiency with chronic i.v. iron supplements induced a statistically significant increase in NGAL values, which reached levels comparable to those described in HD patients with optimal initial iron storage (TSAT ≥ 20%). A similar observation was obtained by others authors [19,20], therefore patients with TSAT levels below

the optimal 'suggested' value of TSAT <20%, with presumed iron scarcity, presented significantly with reduced NGAL levels compared to HD patients with optimal initial iron storage.

NGAL level was found to be closely correlated with S iron, TSAT and S. ferritin. The direct involvement of NGAL in iron balance has been encouraged by the just mentioned observation of rising serum NGAL level in TSAT <20% group after correction of iron deficiency. Both observations corroborate that NGAL level may reflect iron scarcity and iron status in HD patients. Furthermore NGAL showed a good diagnostic power in identifying iron deficiency among HD patients than S ferritin and TSAT.

In the current study, we observed that, hsCRP increased in HD patients and positively correlated with NGAL level. Such observation may denote the role of systemic inflammatory state in up-regulation of NGAL or elevation of NGAL level may be a compensatory defense against the systemic inflammatory state. However NGAL is a true acute-phase factor that can be released by virtually almost every injured tissue [7]. Surprisingly, we also observed that hsCRP was significantly higher in iron deficiency group than non-iron deficiency group, this finding together with lower NGAL levels in an iron deficiency group than non-iron deficiency group, may announce the potential role of NGAL in pathogenesis of iron deficiency anemia in HD patients beyond its role as acute-phase factor. However, this proposition was supported by other researches which hypothesis the role of NGAL in production of iron deficiency anemia in CKD independent of underlying renal function through induction of apoptosis and arrest of differentiation [13]. Finally the debate about the association of chronic inflammatory state, NGAL and anemia in HD patients need further researches.

Conclusion

The potential use of NGAL measurement may be valuable as a novel marker of iron status among hemodialysis patients. The debate about the pivotal role of NGAL and chronic inflammatory state in the pathogenesis of anemia remained to be resolved and whether elevated NGAL is only a marker of CKD and iron status or involved in the pathogenesis of both? The answer of this question needs further researches.

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