Original Research Article

The role of interleukin-6 in anemia of patients with chronic kidney disease, in pre-dialysis stage

Abstract:

Background: Anemia is common in chronic kidney patients. Interleukin-6 plays an important role in anemia. No studies were performed on the role of Interleukin-6 in anemia of pre-dialysis adult chronic kidney disease, so we performed this study to highlight this point.

Methods: A case control study conducted on 50 patients with CKD and 30 apparently healthy volunteers as a control group. They were further subdivided into: 1a: 12 CKD patients with 12 > Hb >10 and 1b: 38 CKD patients with moderate or severe anemia. Hb <10 gm/dl. Group 2: 30 healthy volunteers. All participants were subjected to full history taking, complete clinical examination, routine laboratory investigations including CBC, KFTs, LFTs and serum IL6 assay by ELIZA.

Results: Hb, HCT and MCV were significantly lower among group Ia and group Ib patients in comparison to group II, but CKD patients' groups had significantly higher IL-6 level than control group with no significant difference between patients' groups (group Ia and group Ib). Significant negative correlation was detected between IL6 and both Hb and HCT among patients and control group.

Conclusions: IL6 is significantly higher in chronic kidney patients in comparison to controls. It also correlates negatively with hemoglobin level and hematocrit. IL6 has a good sensitivity of 83.3% at a cutoff value of 74.7 ng/l. So, it can be used as a prognostic marker of anemia in chronic kidney patients.

Keywords: Chronic Kidney Diseases, IL6, Anemia

Introduction

Chronic Kidney Disease (CKD) is a worldwide public health problem. It is more prevalent in the elderly population. However, while younger patients with CKD typically experience progressive loss of kidney function, 30% of patients over 65 years of age with CKD have stable disease [(1)].

According to the Kidney Disease Improving Global Outcomes (KDIGO) guidelines, anemia in adults with CKD is diagnosed when hemoglobin (Hb) concentration is <13.0 g/dl

in males and <12.0 g/dl in females. Many CKD patients have functional iron deficiency, characterized by impaired iron release from body stores which is unable to meet the demand for erythropoiesis [(2)]. Shortened red blood cell survival also contributes to development of anemia. Many data suggest that hepcidin excess may account for the impaired dietary iron absorption and reticuloendothelial cell iron blockade which present in many CKD patients [(3)].

Interleukin-6 (IL-6) also issues a warning signal in the event of tissue damage. Damage-associated molecular patterns (DAMPs), that are released from damaged or dying cells in noninfectious inflammations as burn or trauma, directly or indirectly promote inflammation [(4)]. IL-6 is also involved in the regulation of serum iron level via control of its transporter. IL-6 induces hepcidin production, that blocks the action of iron transporter ferroprotein 1 on gut and thus, decreases serum iron levels. This means that the IL-6-hepcidin axis is responsible for hypoferremia and anemia associated with chronic inflammation [(5)]. Circulating levels of IL-6 inversely correlate with glomerular filtration rate (GFR) in adult patients with CKD. IL-6 is an independent predictor of mortality in adult patients starting peritoneal and hemodialysis [(6)]. Peripheral blood mononuclear cells from hemodialysis patients produced more IL-6 than those from healthy subjects, when stimulated in vitro. IL-6 is elevated in pediatric dialysis patients [(7)]. However, to our knowledge, no studies addressed the role of IL-6 in anemia development in adult pre-dialysis CKD patients. That signifies the importance of this work.

Patients and Methods

This case control study was conducted on 50 patients who had CKD. The patients were recruited from the outpatient clinic and wards of the Internal Medicine Department, Tanta University Hospital. The study duration started from August 2019 to February 2020. Also 30 apparently healthy volunteers were enrolled as a control group. Approval from local ethical committee was obtained (33287/8/19) and a written informed consent was taken from all participants. Participants were divided into the following groups: Group 1 (50 CKD patients), they were further subdivided into group 1a (12 CKD patients with mild anemia 12 > Hb >10) and group 1b (38 CKD patients with moderate or severe anemia, Hb <10 gm/dl). And Group 2 (30 healthy volunteers as a control group).

Inclusion criteria

Patients ≥ 18 years who had estimated glomerular filtration rate (eGFR) 15-60 ml/min/1.73 m^2.

Exclusion criteria

Patients with any of the following conditions were excluded from the study: hemolytic conditions, chronic inflammatory diseases (e.g. rheumatoid arthritis), chronic blood loss (e.g. bleeding hemorrhoids), end-stage renal disease (ESRD) requiring dialysis, eGFR <15 ml/min/1.73 m^2 or malignant disease.

Methods

All the participants were subjected to: Full history taking including: personal history, present history, family history, history of comorbidities and history of drug intake. Clinical examination including: General examination, local abdominal examination.

Investigations including: Routine laboratory investigations including: complete blood count (CBC), aspartate transaminase (AST), alanine transaminase (ALT), serum protein, serum albumin, blood urea, serum creatinine, eGFR, fasting blood glucose, 2 hours post prandial blood glucose and IL 6 assay by enzyme-linked immunosorbent assay (ELISA) using **SUN RED** KIT (**Cat. No.** 201-12-0091 201-12-0091). Serum iron, serum ferritin and parathyroid hormone were measeured in patients only. Pelvi-abdominal ultrasound.:

Sample collection & storage

5 ml of venous blood were withdrawn from each subject under complete aseptic condition, and divided into 1ml collected on EDTA tube for CBC analysis and 4ml collected on a dry vacutainer for the performance of the lab assays they were left to clot, then centrifuged at 3000 g for 10 min. Serum were separated into two separate liquots. The first aliquot was used for lab analysis of aspartate transaminase (AST), alanine transaminase (ALT), serum protein, serum albumin, blood urea, serum creatinine, eGFR, fasting blood glucose, 2 hours post prandial blood glucose, the second aliquot was immediately frozen at -70ºC for analysis of IL6 and avoid repeated freeze-thaw cycles.

ELISA Technique: All reagents and samples were brought to room temperature before use .All standards, controls, and samples were assayed in duplicate.

The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of Human Interleukin 6(IL-6) in samples. Add 6(IL-6) to monoclonal antibody Enzyme well which is pre-coated with Human Interleukin 6(IL-6)monoclonal 6(IL-6) antibody, incubation; then, add Interleukin 6(IL-6) antibodies labeled with biotin, combined with Streptavidin-HRP to form immune complex; then carry out incubation and washing again to remove the uncombined enzyme. Then add Chromogen Solution A, B, the

color of the liquid changes into the blue, And at the effect of acid, the color finally becomes yellow. The chroma of color and the concentration of the Human Substance Interleukin 6(IL-6) of sample were positively correlated

Statistical analysis

Data were collected throughout history. Data were then imported into Statistical Package for the Social Sciences (SPSS version 20.0) for analysis. According to the type of data qualitative was represented as number and percentage, quantitative data were represented as mean ± SD, the following tests were used to test differences for significance. difference and association of qualitative variable by Chi square test. Differences between quantitative independent groups by t test or Mann Whitney, correlation by Pearson's correlation or Spearman's, ROC curve for cutoff value. P value was set at <0.05 for significant results.

Results:

The demographic data of the participants and some laboratory investigations were mentioned in (Table 1). Serum albumin, serum protein and eGFR were significantly lower among patients both groups (group Ia and group Ib) than Group II with no significant difference between patients' groups. Blood urea and serum creatinine were significantly higher among patients' groups (group Ia and group Ib) than group II (control) with no significant difference between patients' groups. No significant difference was detected on comparing FBG and 2h PPG in the studied groups. (Table 1)

Regarding some CBC parameters, hemoglobin (Hb), hematocrit (HCT) and mean corpuscular volume (MCV) were significantly lower in Group Ib when compared to groups Ia and Group II, while white blood cells (WBCs) were significantly higher in Group Ib than in group Ia and Group II, but there was no significant difference regarding platelets count in all groups. As for IL6, it was significantly higher in groups Ia and Ib in comparison to controls, but the difference between group Ia and Ib did not reach statistical significance. (Table 2)

When we studied the levels of serum iron, serum ferritin and parathyroid hormone (PTH) in CKD patients, there was non-significant difference between the two groups. (Table 3)

On studying the correlation between IL-6 and the studied parameters, we found a significant negative correlation between IL6 and Hb, hematocrit (HCT) among CKD patients. On the other hand, the remaining studied parameters showed no correlation with IL-6. (Table 4)

We performed receiver operating characteristic (ROC) Curve for IL-6 which revealed 83.3% sensitivity and 67.7% specificity in detection of anemia in CKD patients at a cutoff value>74.7ng/l of IL-6 with area under the curve= 0.71. (Figure 1)

Table 1: Demographic and some laboratory data of the studied groups.

Parameter	Group I (N=50)		Group II (N=30)	P
Sex (M/F)	25/25		11/19	0.24
Age (years)	52.9±9.33		49.51±7.11	0.091
	Group Ia (N=12)	**Group Ib (N=38)**	**Group II**	**P**
ALT(IU/l)	21.5±8.05	21.79±7.05	19.1±3.56	0.265
	P1=0.917 P2=0.166 P3=0.111			
AST(IU/l)	23.25±7.65	22.42±7.52	26.07±4.21	0.075
	P1=0.743 P2=0.141 P3=0.02			
Serum protien(g/dl)	6.45±2.23	6.01±1.23	7.267±0.54	<0.0001
	P1=0.285 P2=0.005 P3<0.001			
Albumin(g/dl)	3.3±0.65	3.153±0.73	4.27±.74	<0.0001
	P1=0.476 P2<0.0001 P3<0.0001			
Blood urea(mg/dl)	104.1±36.88	116.7±37.65	20.96±6.87	0.0001
	P1=0.452 P2<0.0001 P3<0.0001			
Serum creatinine(mg/dl)	3.183±1.28	3.793±1.24	0.74±0.13	<0.0001
	P1=0.267 P2<0.0001 P3<0.0001			
eGFR(ml/min/1.73m^2)	31.12±16.97	28.51±16.54	134.1±31.16	<0.0001
	P1=0.561 P2<0.0001 P3<0.0001			
FBG (mg/d)	94.58±15.84	92.66±16.3	95.58±14.34	0.688
	P1=0.643 P2=0.679 P3=0.391			
2HPPG (mg/dl)	162.8±71.34	181.3±72.5	124.2±54.3	0.432
	P1=0.739 P2=0.430P3<0.321			

Table 2: Comparison of some CBC parameters and IL-6 level in the studied groups.

Parameter	Group Ia (N=12)	Group Ib (N=38)	Group II	P
Hb (g/dl)	10.15 ±2.03	8.37±1.08	12.4±1.24	0.001
	P1<0.0001 P2<0.0001 P3<0.0001			
Hematocrit (%)	31.84±3.47	25.66±3.32	37.33±3.61	<0.0001

	P1<0.0001 P2<0.0001 P3<0.0001			
MCV (fl)	55.27±9.65	44.94±9.71	80.06±5.13	<0.0001
	P1=0.0008 P2<0.0001 P3<0.0001			
WBCs ($\times 10^3$)/mm ^{3}	8325±2.8	7345±2.5	6447±1.91	0.049
	P1=0.24P2=0.022 P3=0.88			
platelets ($\times 10^3$)/mm^3	298667±92.6	287737±91.6	277400±87.8	0.964
	P1=0.914P2=0.563P3=0.871			
IL6(ng/l)	52.81±29.6	63.73±28.7	34.3±14.89	0.006
	P1=0.654 P2=0.019 P3=0.006			

Table 3: Comparison of serum iron, serum ferritin and PTH levels in CKD patients.

	Group Ia	**Group Ib**	**t**	**P**
Serum iron (µg/dl)	0.693	0.43	1.56	0.125
Serum ferritin (µg/dl)	108.3	55.81	1.92	0.061
PTH (pg/ml)	198.2	192.4	10.262	0.795

Table 4: Correlation of IL-6 with the studied parameters in CKD patients.

Parameter	**IL-6 in CKD patients**	
	r	P
Hb(g/dl)	-0.333	0.003
Hematocrit (%)	-0.286	0.043
MCV (fl)	-0.144	0.320
MCH (pg)	0.219	0.126
Iron(µg/dl)	0.004	0.976
Ferritin(µg/dl)	0.023	0.876
WBCs ($\times 10^3$) /mm ^{3}	0.134	0.355
PLTs ($\times 10^3$) /mm ^{3}	0.059	0.685
Cr(mg/dl)	0.134	0.352
eGFR(ml/min/1.73m^2)	0.007	0.961
PTH (pg/ml)	0.185	0.197

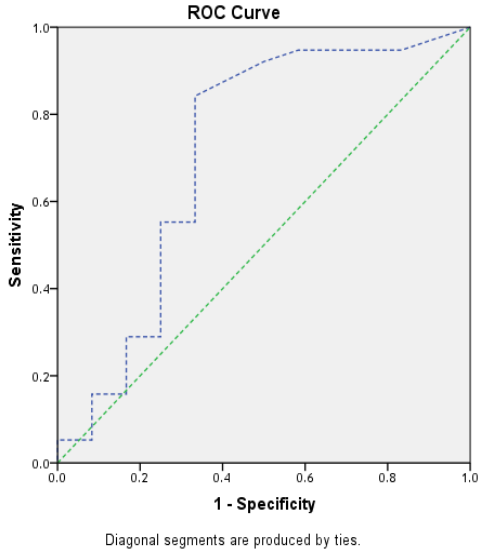

Figure 1: ROC Curve for of IL-6. At a cutoff value>74.7ng/l of IL-6 it had 83.3% sensitivity and 67.7% specificity

Discussion

Chronic kidney disease (CKD) is a worldwide public health problem. CKD is more prevalent in the elderly population. However, while younger patients with CKD typically experience progressive loss of kidney function, 30% of patients over 65 years of age with CKD have stable disease [(1).]

However, very few studies have evaluated IL-6 in adults with pre-dialysis CKD, so this study was designed to study the role of interleukin 6 in anemia of pre-dialysis chronic kidney disease.

This study was conducted on 50 patients who had CKD, in addition to 30 healthy volunteers who were enrolled as a control group. Patients were recruited from the outpatient clinic and wards of the Internal Medicine Department, Tanta University Hospitals during the period from August 2019 to February 2020.

In the current study, the mean hemoglobin level in groups Ia and Ib were significantly lower in comparison to controls. The same findings were recorded as regard the hematocrit.

Raj et al.[(8)] reported that 76% of their studied CKD patients had anemia compared to 6.7% of controls. They attributed that to inadequate synthesis of erythropoietin mediated by

renal insufficiency and the anti-proliferative effects of accumulating uremic toxins. Those were the primary cause of anemia in ESRD.

The mean IL6 in group Ia patients was 52.81±29.6 ng/l, 63.73±28.7 ng/l in group Ib patients and 34.3±14.89 ng/l in control group with significant difference, this significant difference between patients and controls regarding IL6 may be responsible for the high prevalence of anemia among cases.

Inflammation leads to IL-6 synthesis by macrophages, which acts on hepatocytes and induce hepcidin production [(9)].

On the other hand, in **Ewelina et al. 2015[(10)]** study, they did not confirm statistically significant difference between IL-6 concentration in patients with and without iron deficiency. However, IL-6 level tended to increase as kidney function was impaired only in the group with absolute iron deficiency.

In the study by **Shu et al**.[(11)]**,** IL-6 increased in patients with tumor related anemia, but there was no correlation between IL-6 and hepcidin in patients with iron deficiency anemia. They suggested that iron deficiency may have more significant impact on reducing hepcidin levels than the inflammation itself.

However, in healthy Japanese adults **Nakagawa H et al**.[(12)] showed that lower iron concentration was associated with increased serum IL-6.

In agreement to our results, **Przybyszewska et al**.[(13)] revealed a significantly higher concentration of IL-6 in patients with anemia of chronic disease in comparison to patients with iron deficiency anemia.

Inflammatory cytokines, including IL-6, appear to blunt the response to erythropoietin in patients with ESRD [(14)].

Barreto et al. 2010[(15)]. illustrated that in the elderly and CKD patients on dialysis, IL-6 levels tended to rise as CKD progressed with the increase becoming statistically significant at CKD stages 5.

A study conducted by **Malaponte et al**.[(16)] showed that the ability of hemodialysis patients to secrete tumor necrosis factor (TNF-a), interleukin-1 (IL-1b) and IL-6 by

stimulated monocytes decreased progressively according to length of dialysis therapy. However, the fact that spontaneous production of IL-6 tended to be already elevated in hemodialysis patients suggests that the monocytes might be chronically activated and subsequently refractory to any further stimulation**.**

Zhang et al.[17] suggested that measuring baseline inflammatory markers could provide information about outcome. Some researchers also suggested monitoring their time-course oscillations. Although the association between longitudinal inflammatory variation and risk prediction has only been examined in a few studies, the repeated elevations of IL-6 may be more predictive than a single elevation [18].

In agreement with our study, **Oberg et al**.[19] evaluated plasma IL-6 levels in patients at earlier CKD stages (3–5) found that this interleukin was significantly elevated in the CKD patients (compared with healthy controls), but that there was no association with the estimated glomerular filtration rate.

As for the correlation of IL-6 with the studied parameters, we found significant negative correlation between IL-6 and both hemoglobin and hematocrit, while no significant correlation could be detected with the rest of the parameters.

This was in agreement with **Barreto et al. 2010** study who reported a significant relationship between IL-6 and Hb level ($P<0.005$). They also reported no significant relationship between IL-6 level and PTH [15].

Our results were contradictory to those reported by **Barreto et al,** who confirmed an inverse linear relationship between IL-6 levels and the eGFR when the analysis was restricted to pre-dialysis CKD patients at stages 2–5 [15].

To further identify the role of IL-6 in anemia in CKD patients, we performed the ROC curve, which demonstrated a sensitivity of 83.3% and a specificity of 67.7% at a cutoff value for IL-6 of >74.7 ng/l.

Conclusion

Anemia is a common finding in patients with chronic kidney disease. IL6 is significantly higher in CKD patients in comparison to controls. It also correlates negatively with hemoglobin level and hematocrit. IL6 has a good sensitivity of 83.3% at a cutoff volume of 74.7 ng/l. So, it can be used as a prognostic marker of anemia in CKD patients. Il-6 could also serve as a therapeutic target in anemic CKD patients.

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