Serum interleukin-6 is associated with hypocalcemia, hypoferritinemia and hyperkalemia in end- stage renal disease patients

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ABSTRACT

Both of chronic inflammation and mineral disturbance are major concerns in patients with chronic kidney disease, particularly end-stage renal disease (ESRD). The present study aimed to investigate the association between circulating interleukin-6 (IL-6) and minerals dysregulation in patients diagnosed with ESRD and on a continuous hemodialysis regimen. This cross-sectional study included 74 patients undergoing continuous hemodialysis. Serum samples were tested for IL-6 using an enzyme-linked immunosorbent assay. Mineral were analyzed using an electrolyte analyzer and biochemical tests. Parameter correlations were analyzed using the Pearson's correlation test. Among the studies group, the male: female ratio was 1:0.72. IL-6 mean value was 13.77 pg/mL ±9.79 SD. IL-6 was significantly negatively correlated with circulating iron and calcium levels (r= –0.229, P=0.049; r= –0.252, P=0.03, respectively). IL-6 was significantly positively correlated with K^+ levels (r=0.269, P=0.02). The present study highlighted the substantial role of IL-6 in mineral dysregulation in hemodialysis patients. However, identifying IL-6 as potential therapeutic target for minimizing and monitoring clinical effects of mineral disturbances in hemodialysis patients still requires further confirmatory studies.

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Introduction

Chronic kidney disease (CKD) is an increasing health issue, affecting 10-12% of the population worldwide. Chronic inflammatory status is a broadly disseminated condition in category.[1] Chronic inflammation is a significant consequence of end-stage renal disease (ESRD) and contributes to an increased risk of metabolic disorders such as mineral disturbance. Between 30-50% of patients with ESRD have increased levels of inflammatory markers, interleukin (IL)-6, IL-1, tumor necrosis factor-α (TNF-α), and C-reactive protein (CRP).[2]

Several factors are associated with the development of the inflammatory milieu, including catheters, dialysis process itself, dysautonomia, and increased epithelial intestinal dysfunction that causes increased intestinal permeability, dysbiosis, and bone mineral disturbance. This inflammatory milieu is further heightened by the accumulation of uremic toxins due to insufficient clearance by hemodialysis process.[3,4]

Hemodialysis is an important technology implicated in reducing risks of morbimortality and saving the lives of patients with progressive renal failure. However, it is still suboptimal and contributes to the chronic systemic inflammatory state.[5]

Patients with progressive renal failure experience significant mineral dysregulation. Overthrown mineral

metabolism contributes to clinical symptoms, such as vascular calcification, bone demineralization, muscle atrophy, sexual impairment anemia and neuropathy.[6,7]

Proinflammatory cytokines result in renal cell dysfunction in a receptor-mediated pattern, function of trans-epithelial transporters, and ion channels along nephron cells.[8] Under normal physiological conditions, IL-6 plays a protective role; however, when its serum level increases, it plays a pathogenic role. IL-6, IL-3 IL-1, and TNF-α have been reported to mediate hypercalcemia in patients with active myeloma.[9]

Limited information is available regarding the effect of IL-6 on mineral metabolism in patients with CKD. The current study aimed to evaluate the serum levels of IL-6 in ESRD patients on continuous hemodialysis and verify its correlation with mineral dysregulation.

Materials and Methods

This single-center, cross-sectional study was conducted from November-2021 to February 2022 at the Hemodialysis Unit, Immamain Alkadhymain Medical City, Baghdad, Iraq. A total 74 patients with ESRD undergoing chronic hemodialysis were enrolled in this study. ESRD was defined as an estimated glomerular filtration rate (eGFR) less than 15 mL/min/1.73 m^2 of body surface.[6] Only patients who fulfilled the following criteria were considered for participation in the present study: i) patients who did not receive other renal replacement therapies; ii) patients were clinically stable and serologically negative for human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV) infections and apparently did not show any evidence of bacterial infection; iii) patients who did not record malignant disease; iv) patients who did not receive immunosuppressant drugs; v) patients commenced continuous hemodialysis. The dialysis regimen consisted of three sessions' weekly for 4 hours. for each session. Blood samples were collected from the patients prior to the dialysis session. IL-6 levels were tested using ELISA (Raybiotech/USA). Mineral Na^+, K^+ and CL^- were analyzed using an electrolyte analyzer, while Fe^{++}, Ca^{++} and PO_4^- were tested using biochemical tests (Numedica/UK). Other laboratory information was collected from the patient records.

Ethical approval

The study protocol did not interfere with medical prescriptions and recommendations. This study was approved by the Institutional Review Board of the College of Medicine, AL-Nahrain University (202206166). All patients provided written informed consent before enrollment in the study. For the children, informed consent was provided by the parents.

Statistical analysis

Statistical analysis was conducted using the Statistical Package for the Social Sciences Software Version 23 (SPSS 23) and Excel 2013. Sample size was determined according to 90% confidence level and 5% confidence interval. Categorical data were expressed as frequency and percentage. Continuous variables such as baseline and laboratory data were expressed as median (range) and mean ± standard deviation (SD). The correlation of parameters was analyzed using the Pearson's correlation test. Patients with missing data were excluded from the study.

Results

A total of 74 patients who underwent continuous dialysis, thrice weekly for 4 hours per session, were enrolled in this study in order to verify mineral dysregulation in ESRF patients and to determine any correlation between dysregulation and IL-6. The mean age of the participants was 50.14±15.96 year. Most of them were age group ranging 60-79 year. The male to female ratio was 1:0.72. Patient baseline characteristics are shown in Table 1. Mean value of circulating IL-6 among patients with ESRF was 13.77±9.79 SD pg/mL, other laboratory characteristics of patients are shown in Table 2.

The correlation between minerals and acute-phase proteins was also studied. The results showed no significant correlation between the studied minerals, Ca, Na, Cl, PO_4, K, and Fe, and the acute phase proteins, ferritin, albumin, and hepcidin (Table 3).

The correlation between IL-6 and minerals was also investigated. The results revealed that IL-6 had a significant negative correlation with both Fe^{++} and Ca^{++} and a significant positive correlation with K^+ (r= –0.229, P=0.049; r= –0.252, P=0.03, and r=0.269, P=0.02, respectively, as shown in Figure 1. In contrast, IL-6 did not show any correlation with other minerals, Na^+, r= –0.076, P=0.518; CL^-, r=0.138, P=0.241; and PO_4^-, r=0.082, P=0.488 (Figure 1).

Discussion

Although limited, several studies have pointed to the importance of mineral dysregulation and inflammation as substantial factors for increased morbidity and mortality in ESRD patients;[10] however, the interrelation between these factors has barely been discussed.

The current cross-sectional study aimed to investigate the correlation between IL-6, minerals, and acute-phase proteins in patients with ESRD.

Chronic inflammation is a fundamental factor in the pathogenesis of CKD and is the most severe stage of ESRD. Accumulated toxins in the circulation result in oxidative stress and accumulated reactive carbonyls that

Table 1. Baseline characteristics of participants.

Variable		Median (range)	Mean±SD
Age (year)		54.5 (12-78)	50.14±15.96
Duration of chronic dialysis (year)		3 (0.83-6)	3.21±1.15
		Number (percent)	
Age group	1-19	5 (6.75%)	
	20-39	14 (18.91%)	
	40-59	25 (33.78%)	
	60-79	30 (40.54%)	
	80≥	0 (0%)	
Gender	Female	31 (41.9%)	
	Male	43 (58.1%)	
Dialysis modality	D	14 (18.9%)	
	F	60 (81.1%)	
Primary renal disease	HT	24 (32.4%)	
	DM	18 (24.3%)	
	Congenital	14 (18.9%)	
	Shock	6 (8.1%)	
	PROU	3 (4.1%	
	Unknown	9 (12.2%)	

D, double lumen; F, fistula; HT, hypertension; DM, diabetes mellitus; PROU, prostate operating unit.

Table 2. Laboratory characteristics of participants.

Variable	Median (range)	Mean±SD
Blood urea (mg/dL)	126.5 (45.2-223)	130.92±41.32
S. creatinine (mg/dL)	6.1 (2.88-12.88)	6.23±1.68
Hb (g/dL)	9.2 (5-13.2)	9±1.72
S. albumin (g/dL)	3.43 (1.92-5.3)	3.47±0.39
S. ferritin (ng/mL)	587 (11.43-1200)	622.36±355.01
Hepcidin (ng/mL)	4.71 (0.71-16.47)	5.65±3.83
Blood glucose (mg/dL)	112.5 (65.15-387.61)	133.07±62.07
TSB (mg/dL)	1 (0.15-56)	1.72±6.41
S. GOT (A)	13.65 (4.3-195)	16.8±22.29
S. GPT (A)	11.1 (0.8-41)	12.19±8.42
S. ALP (A)	302.75 (163-1793)	423.32±342.4
S. total protein (g/dL)	6.4 (4.31-11)	6.48±0.83
S. Na^+ (mmol/L)	137 (92.3-144)	135±9.09
S. iron (µg/dL)	40 (1.04-112.5)	45.32±24.14
S. K^+ (mmol/L)	5.09 (4-6.95)	5.15±0.6
S. Cl^- (mmol/L)	102.8 (92-135)	102.94±6.75
S. Ca^{2+} (mg/dL)	9.5 (5.77-13)	9.23±1.22
S. PO_4 (mg/dL)	6.07 (3.04-14)	6.03±1.81
S. Vit. D (ng/mL)	10.75 (8-106)	14.14±12.3
S. PTH (pg/mL)	192.5 (27.4-1500)	289.7±328.91
S. IL-6 (pg/mL)	11 (6.31-68.24)	13.77±9.79

S, serum; Hb, hemoglobin; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; ALP, alkaline phosphatase; PTH, parathyroid hormone; IL-6, interleukin-6.

would result in a massive proinflammatory status. During the hemodialysis process, blood comes into contact with dialyzing membranes, activating a cascade of events culminating in the release of proinflammatory mediators into the surrounding milieu. Furthermore, renal dysfunction leads to impaired proinflammatory cytokines clearance.[11,12]

In the current study, IL-6 circulating level revealed a negatively correlated with circulating iron levels. r= –0.229, P=0.049. There is a major perspective that inflammatory cytokines such as IL-6 play substantial role in hyperferritinemia that correlates to anemia of chronic diseases.[7] This is thought to be mediated by hepcidin, a type II acute phase protein. Hepcidin is a master regulator of iron metabolism, reduces intestinal iron absorption, and causes sequestration of iron in macrophages, thus potentially inhibiting iron transport to erythrocytes in the bone marrow and consequently resulting in erythropoiesis suppression.[13]

Several *in vitro* and *in vivo* studies have revealed that the inflammatory mediators IL-1β, IL-1α, and IL-6 enhance hepcidin mRNA synthesis.[14] Another study documented that crosstalk between IL-6 *via* the JAK/STAT and iron-signaling pathways in hepatocytes mediates the regulation of hepcidin production; however, the exact mechanism is still under investigation.[15]

It has also been reported that IL-6 reveals a negative correlation with calcium levels. r= –0.252, P=0.030. It can be said that IL-6 decreases serum calcium indirectly. IL-6 increase causes deficiency in both vitamin D and parathyroid hormones (PTH). These two factors reduce the body calcium levels. Vitamin D enhances the expression of calbindin, an intracellular calcium transporter, and transports calcium from the intestinal lumen

Table 3. Correlation of minerals with acute phase proteins.

Acute phase protein		Ca mg/dL	Na mmol/L	Cl mmol/L	PO_4 mg/L	K mmol/L	Fe µg/dL
Ferritin (ng/mL)	r	–0.021	–0.195	0.164	–0.006	0.123	0.189
	P	0.856	0.097	0.162	0.961	0.297	0.106
Albumin (g/dL)	r	–0.169	0.218	–0.216	–0.055	0.001	0.073
	P	0.151	0.062	0.064	0.642	0.294	0.538
Hepcidin (ng/mL)	r	0.162	–0.103	0.157	–0.034	0.174	0.088
	P	0.168	0.381	0.182	0.775	0.138	0.454

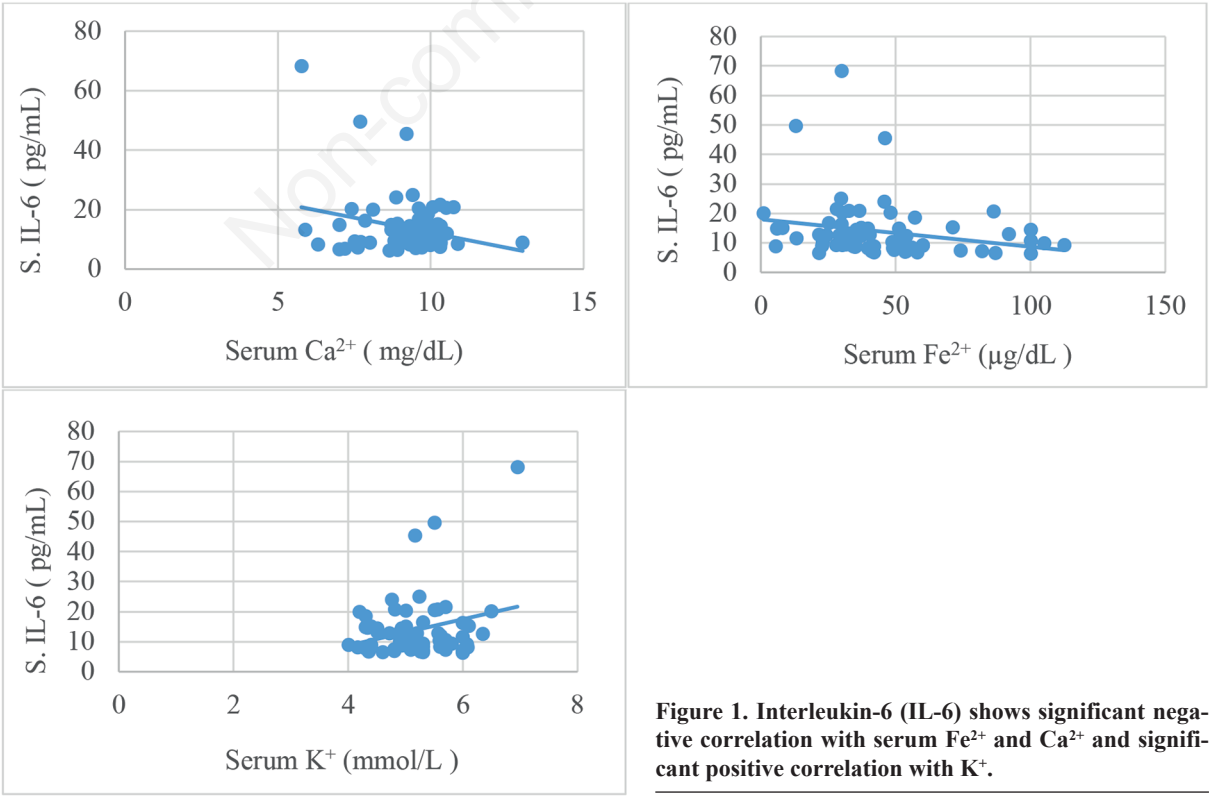

Figure 1. Interleukin-6 (IL-6) shows significant negative correlation with serum Fe^{2+} and Ca^{2+} and significant positive correlation with K^{+}.

through the epithelium into circulation. Vit-D also stimulates osteocalcin synthesis in osteoblasts. Osteocalcin is released in the bone marrow microenvironment and undergoes conformational changes, resulting in its alignment with calcium ions in the form of hydroxyapatite, leading to bone formation.[16]

PTH promotes vitamin D renal synthesis and increases calcium reabsorption in three locations: the Henle-ascending loop, distal convoluted tubule cells, and collecting duct. Furthermore, PTH binds to its receptors on osteoblasts and osteocytes, which pumps calcium from the bone marrow into the circulation.[17] Thus, Vit-D and PTH deficiencies may disturb calcium homeostasis in the body.

The current study pointed to a significant positive correlation between IL-6 and serum potassium. r=0.269, P=0.020. It is well established that renal drug transporters and ion channels, such as renal tubular potassium channels, which spread along the nephron, are affected by proinflammatory cytokines in receptor-mediated pattern.[16]

In uremic environment, the proinflammatory milieu affects renal potassium channel potential that would result in disturbance of the physiological tubular transport and cause renal cell injury. TNF-α was reported to disturb potassium channel activity that induced apoptosis, such prolonged effect ultimately results in animal sudden cardiac attack.[8] In accordance with that, an *in vitro* study conducted on fibroblasts treated with TNF-α and IL-1β, showed an increase in gene expression of large-conductance K^+ (BK channel) subunits KCNMA1and KCNMB3.[18]

Consistent with our results, other studies have revealed that cytokines affect potassium transport. TGF-β1 has been documented to upregulate the activity of renal 'stretch activated potassium-channel' by enhancing their mRNA expression.[8] An *in vitro* study reported the biphasic receptor-mediated effect of INF-y on 40-pS potassium channel activity in a time-dependent manner, acute stimulatory effect, in contrast to delayed inhibitory effect.[8] It is believed that at least in part, proinflammatory cytokine effects on renal cells are mediated via the activation of certain transcription factors that initiate the synthesis of effector proteins such as adhesion molecules and nitric oxide species.[19] These factors, in turn, affect potassium transport activity, indirectly. IL-6 is well contributed to oxidative stress, it is worth noting that potassium current activation is an early response to pro-oxidants over production.[20]

Although some proinflammatory cytokines were reported to activate gene synthesis of BK-channel, others were recorded to reduce it.[8] Therefore, further studies are required to demonstrate the effect of the complete panel of the proinflammatory cytokines on potassium channel activity, other ion transporters and minerals that would mightily minimize minerals dysregulation in ESRD patients and consequently reducing the relevant clinical adverse effects.

The present study had some limitations. It included smaller size number of patients due to the fact that not all patients are restricted to three session per week, mainly due to their low financial, educational and awareness status. Patients on the two hemodialysis pattern (double lumen catheter and arteriovenous fistula) were gathered in one group, no subgrouping was applied, due to the low number of patients on double lumen hemodialysis that cannot be statistically analyzed.

Conclusions

Circulating IL-6 levels had a substantial impact on mineral disturbances. In the present study, IL-6 showed a significant negative correlation with circulating iron and calcium levels and a significant positive correlation with circulating potassium levels. With the growing number of patients with renal diseases, this study represents an update to the present knowledge about IL-6 pathogenesis in ESRD in terms of mineral dysregulation. However, monitoring blood IL-6 in aim to adjust the potential pathologic role of mineral disturbances in hemodialysis patients still requires further controlled studies that involve larger number of participants. Such studies would elaborately evince the potential role of IL-6 regulation in controlling the adverse effect of minerals dysregulation in patients with continuous hemodialysis, such as cardiovascular and neurological complications.

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