Inflammatory and Nutritional Biomarkers: Role as Non -Traditional Risk Factors for Cardiovascular Morbidity in Patients with Chronic Kidney Disease

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Abstract: ***Introduction:*** Chronic kidney disease (CKD) patients have an increased cardiovascular (CV) risk that cannot be explained completely by traditional cardiovascular risk factors (CVRFs). Chronic low grade inflammation is common among patients with renal disease and probably contributes to cardiovascular disease (CVD). Moreover, the prevalence of protein energy wasting (PEW) among patients with CKD is high and is associated with a proinflammatory state. Malnutrition, inflammation, and atherosclerosis often coexist among patients with CKD, and each of these risk factors independently predicts outcome in these patients. ***Aim of the work:*** This study was designed to assess inflammatory and nutritional biomarkers in conjunction with echocardiographic assessment in CKD patients on hemodialysis and on conservative treatment in order to clarify the role of inflammatory and nutritional biomarkers as risk factors for CV complications in these patients.***Patients and methods:*** This study was carried out on 70 subjects: 30 CKD patients on regular hemodialysis, 20 CKD patients on conservative treatment and 20 healthy subjects. All cases were subjected to history taking, full clinical examination, ECG, echocardiography and carotid duplex. Hemoglobin level and serum level of creatinine, urea, albumin, cholesterol, triglycerides, HDL, LDL, iron, ferritin, CRP, IL-1ß and IL-18 were measured. ***Results:*** There was a significant increase in the level of inflammatory markers: CRP, IL-1ß and IL-18; and a significant decrease in the level of nutritional factors: albumin, iron and ferritin in both patient groups versus the controls and in group I versus group II. Regarding the echocardiographic data, there was a statistically significant difference in fractional shortening (FS), ejection fraction (EF), posterior wall thickness (PWT), inter ventricular septum thickness (IVST) and intima-media thickness (IMT) in both patient groups compared to the control group.A positive correlation was found between inflammatory factors (CRP,ILß and IL18) and urea and creatinine while there was a negative correlation between nutritional factors(albumin, iron, ferritin and hemoglobin) with urea and creatinine with a negative correlation between inflammatory factors and nutritional factors .***Conclusion:*** CKD patients especially those on HD, should be considered at high risk for developing CVD. The elevated levels of proinflammatory markers are associated with CV morbidity and may contribute to the deterioration of nutritional status in end stage renal disease (ESRD). Thus, it could be speculated that suppression of the vicious cycle of malnutrition, inflammation and atherosclerosis would improve survival in dialysis patients.

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1. Introduction

Chronic kidney disease (CKD) patients have an increased cardiovascular (CV) risk that cannot be explained completely by traditional cardiovascular risk factors (CVRFs). Non-traditional CVRFs such as oxidative stress, abnormal calcium and phosphate metabolism, hyperhomocysteinaemia, malnutrition and "inflammation syndrome", represent novel therapeutic targets for clinical interventions in this patient population[1].

Chronic low grade inflammation is common among patients with renal disease and probably contributes to cardiovascular disease (CVD)[2]. Moreover, the prevalence of protein energy wasting (PEW) among patients with CKD is high and is associated with a proinflammatory state[3-5]. Malnutrition, inflammation, and atherosclerosis often coexist among patients with CKD, and each of these risk factors independently predicts outcome in these patients[6].

The causes of inflammation in CKD are definitely multifactorial[7]. Low- grade infection, repeated exposure to dialysis filters and auto-oxidation products are considered as likely inciting factors in these patients[8]. Furthermore, a variety of traditional and non-traditional risk factors such as sympathetic hyperactivity, dyslipidemia, hyperphosphatemia/ hyperparathyroidism, diabetes and smoking may activate and/or amplify the inflammatory process in end-stage renal disease (ESRD)[9]. Available data suggest that pro-inflammatory cytokines may play a central role in the genesis of the metabolic syndrome[7].

Atherosclerosis is the main cause of morbidity and mortality in patients with ESRD and there is consistent evidence that C-reactive protein (CRP)

and proinflammatory cytokines such as interleukin-1ß (IL-1ß), interleukin-6 (IL-6) and tumor necrosis factor- α (TNF-α) are risk factors for atherosclerotic cardiovascular outcomes in these patients[10-11].

CRP, a nonspecific marker of inflammation, is the most important factor in the inflammatory syndrome, and its production is controlled by several inflammatory mediators including IL–6 which is the main cytokine in the acute phase of inflammation. CRP and serum albumin levels can predict mal-nutrition, arthrosclerosis, cardiac diseases, and death in ESRD patients[12].

IL-1ß, which is a major proinflammatory cytokine, may further amplify inflammation and lead to malnutrition, through inducing anorexia, and muscle wasting due to increased protein breakdown. Several clinical studies have shown that the circulating level of IL-1ß may affect nutritional status, especially body composition. Although a number of factors are related to malnutrition and wasting in ESRD, pro-inflammatory cytokines, such as IL-1ß, may play an important role.[13]

Whitman and his colleagues in 2002[14] pointed out that interleukin-18 (IL-18) is a novel proinflammatory cytokine that promotes atherosclerosis and plaque vulnerability in experimental models.

PEW is highly prevalent (34-65%) in CKD, and is considered among the strongest predictors of death in this patient population. Although many factors contribute to impaired nutritional status in CKD patients, inflammation is one of the most important factors. Furthermore, PEW and inflammation are strongly interrelated in the clinical setting[5,15-16]. Hypoalbuminemia, which is common in CKD patients and usually consequent to PEW, is strongly associated with inflammation. Many studies demonstrated that CRP was the primary predictor of serum albumin levels in hemodialysis (HD) patients. Increase in the levels of proinflammatory cytokines is related to high prevalence of PEW and hypoalbuminemia in CKD patients[17-19].

Although serum ferritin is an imperfect marker of inflammation, low levels have been shown to correlate well with iron deficiency and high levels are more likely to correlate with inflammation[20].

Inflammation is closely related to PEW in dialysis patients and the simultaneous combination of these two conditions, also referred to as 'malnutrition–inflammation complex syndrome' (MICS), is observed frequently in dialysis patients[21]. While MICS may play a central role in poor clinical outcome including a high rate of mortality and hospitalization and diminished quality of life, it may also lead to hyperferritinaemia and refractory anemia including erythropiotein hyporesponsiveness in these individuals[22]. It is not clear whether PEW alone or combined with inflammation in the form of MICS has a significant effect on serum ferritin in HD patients[23].

Aim of the work:

This study was designed to assess inflammatory and nutritional biomarkers simultaneously in patients with CKD, either on HD or conservative treatment, in conjunction with echocardiographic and carotid duplex assessment in order to clarify the role of inflammatory and nutritional biomarkers as risk factors for CV complications in CKD patients.

2. Patients and Methods:

This study was carried out on 70 subjects divided into three groups:

Group I: Including 30 patients with CKD on regular HD, 3 times per week in 4 hours sessions. They were 17 males (56.7%) and 13 females (43.3%).

Group II: Including 20 patients with CKD on conservative treatment. They were 10 males (50%) and 10 females (50%).

The etiology of renal failure was variable among the two studied patient groups.

Group III: Including 20 age and sex matched healthy subjects as a control group . They were 10 males (50%) and 10 females (50%).

Informed written consents were obtained from all patients according to the Declaration of Helsinki.

All patients and controls in this study were subjected to the following:

A. History taking: laying stress on symptoms of cardiac complications e.g. previous anginal episodes, thrombotic events, ECG documented arrhythmia etc.

B. Clinical examination: to confirm the diagnosis and to detect signs of CV complications, measurement of arterial blood pressure and pulse .

C- Electrocardiogram (ECG).

D-Echocardiography: Standard transthoracic M-mode, two dimensional, continuous and pulsed wave Doppler echocardiograms were obtained soon after a session of routine HD using 2.5 MHz transducer. Pulsed and continuous Doppler color coded duplex examination was used to evaluate both diastolic cardiac functions and the functional efficacy of the cardiac valves. M-mode measurements were used to evaluate the left ventricular posterior wall thickness and left ventricular internal dimensions both in systole and diastole aiming to calculate the fractional shortening (FS) and ejection fraction (EF).

E-Carotid Duplex: Ultrasonographic studies on common carotid arteries were performed by using a 7.5 MHz high resolution probe. The intima-media thickness (IMT) was defined as a low-level echo gray band that does not project into the arterial lumen and was measured during end-diastole as the distance from

the leading edge of the second echogenic line of the far walls of the distal segment of the common carotid artery, the carotid bifurication and the initial tract of internal carotid artery on both sides.

F- Laboratory Investigations: Blood sampling was performed after a 12-hrs fast. In HD group blood samples were obtained before the first session of the week. Ten ml venous blood was obtained by clean venipuncture from the antecubital vein and divided as follows: 2 ml into EDTA anticoagulated vacuum tube for complete blood picture and 8 ml into a plain vacuum tube, serum was separated after blood clotting by centrifugation. Part of freshly separated serum was used for routine chemistry done on the collection day namely: serum creatinine, urea, albumin, , serum cholesterol, triglycerides, high density lipoprotein (HDL),low density lipoprotein(LDL) cholesterol, calcium and phosphorus. These tests were performed using auto analyzer Beckman CX3 Delta analyzer.

The rest of the separated serum was stored at -20°C for further determination of inflammatory markers (CRP, IL-1ß and IL-18) and nutritional markers (iron and ferritin).

* **Serum CRP** determination was done using immunoturbidimetric assay (Randox laboratories, Ltd). Kon progress/specific.

* **Serum IL-1ß** levels were measured using the Quantikine High Sensitivity Human IL-1ß ELISA kit from R&D Systems, Inc, Minneopolis, Minnesota, USA (sandwich immunoassay).

* **Serum IL-18** levels were determined using the MBL International Corporation Human IL-18 ELISA kit from R&D Systems Inc, Minneopolis, Minnesota, USA. (a quantitative sandwich immunoassay technique).

* **Quantitative determination of serum iron** was performed by functional chromogenic assay using Stanbio Iron kit (Stanbio Laboratory Inc,USA).

* **Serum levels of ferritin** were determined by enzyme immunoassays using Pathozyme-Ferritin kit (Omega Diagnostics Limited, UK).

All assays were carried out according to manufacturers' instructions.

Statistical methods

Data were summarized as mean values± SD. The ANOVA test was employed for intergroup comparison. Association between variables was assessed by Pearson correlation coefficient. The threshold for significance was a *P*-value ≤ 0.05. Statistical analysis was performed with the aid of the SPSS computer program, version 16.

3. Results

The demographic and clinical data of patients and controls are shown in table 1. There was statistically significant increase in systolic blood pressure in both groups I and II compared to the control group.

Regarding the echocardiographic data, there was a statistically significant decrease in fractional shortening (FS) and ejection fraction (EF) and a significant increase in end systolic dimension (ESD), posterior wall thickness (PWT), inter- ventricular septum thickness (IVST) and intima-media thickness (IMT) in groups I and II in comparison to the control group (table 2).

The results of routine laboratory investigations are illustrated in table 3 while the results of inflammatory and nutritional markers are shown in table 4. The serum levels of CRP, IL-1ß and IL-18 showed statistically significant increase in group I and group II in comparison to the control group together with a statistically significant increase in group I compared to group II. However, there was a statistically significant decrease in serum iron, ferritin, hemoglobin and albumin in both groups (I&II) compared to the control group and also a statistically significant decrease in group I compared to group II.

The results of correlation analysis are shown in table 5 .

Table (1): Demographic and clinical data of studied groups. (mean values± SD)

	Group I (HD) (n=30)	Group II Conservative (n=20)	Group III (Controls) (n=20)
Age (years)	55.8±8.3	54.7±14.2	51.3±10.9
Gender M F	17(56.7%) 13(43.3%)	10(50%) 10(50%)	10(50%) 10(50%)
Systolic BP	132.6±21.3*	139.0±21.2*	118.3±8.1
Diastolic BP	83.0±11.4	87.5±12.5*	78.7±4.2
Pulse (beat/min)	84.3±7.7	84.8±13.2	79.2±10.2
Duration of dialysis (years)	5.6±3.5		

* Highly significant difference *vs* controls $p< 0.01$.

Table (2): The echocardiographic data of studied groups(mean values± SD)

	Group I (HD)	Group II (Conservative)	Group III (Controls)
EDD (mm)	55.0±8.8	55.2±6.6	51.4±7.1
ESD (mm)	36.6±8.9*	36.9±9.0*	31.1±5.3
FS (%)	34.5±7.7*	34.4±5.6*	39.3 ±5.7
EF(%)	61.5±10.5**	58.8±14.7**	69.1±7.3
PWT (mm)	10.9±2.4**	10.7±3.0*	8.8±1.3
IVST (mm)	11.2±2.6**	9.9±2.4*	8.8±1.1
IMT (cm)	1.1±0.19**	1.0±0.22**	0.71±0.88

*Significant difference *vs* controls $p< 0.05$.
** Highly significant difference *vs* controls $p< 0.01$.
EDD: end diastolic dimension, ESD: end systolic dimension, FS: fraction shortening, EF: ejection fraction, PWT: posterior wall thickness, IVST: inter ventricular septum thickness, IMT: intima-media thickness.

\Table (3): Results of routine laboratory investigations in studied Groups (mean values± SD)

	Group I (HD)	Group II (Conservative)	Group III (Controls)
Urea (mg/dl)	139.1 ±28.6*	125.5±47.3*	30.4±4.3
Creatinine (mg/dl)	8.0±1.9*#	3.6±2.0*	1.2±0.2
Ca (mg/dl)	9.1 ±1.2	9.0±1.1	9.6±0.6
PO_4 (mg/dl)	4.7±1.7*	5.3±1.2*	3.3±0.8
Cholesterol (mg/dl)	183.3 ±56.8	181.1±67.4	192.5±15.4
Triglycerides (mg/dl)	245.1±110.7*	202.2±109.5	153.1±13.8
HDL (mg/dl)	34.6 ±21.2*	34.6±11.6*	51.1±15.9
LDL (mg/dl)	105.1 ±51.7	106.0±58.3	110.7±28.2
Hemoglobin (g/dl)	8.5±0.45*#	10.3±0.4*	13.7±0.51

* Highly significant difference *vs* controls $p< 0.01$.
#Highly significant difference *vs* group II $p<0.01$.
Ca: calcium, PO_4: phosphorus, HDL: high density lipoprotein cholesterol, LDL: low density lipoprotein cholesterol.

Table (4): Results of inflammatory and nutritional markers in studied groups(mean values± SD)

	Group I (HD)	Group II (Conservative)	Group III (Controls)
CRP (mg/l)	7.2±2.7*##	4.8±0.9*	1.8±0.4
IL-1ß (pg/ml)	42.5±8.8*##	25.5±5.9*	9.4±2.2
IL-18 (pg/ml)	502.2±99.2*##	307.9±67.7*	123.8±37.4
Iron (ug/dl)	34.8±4.7*##	57.6±16.05*	101.5±13.5
Ferritin(ng/ml)	40.6±6.2*##	57.2±13.5*	117.9±23.5
Albumin (g/dl)	2.9 ±0.5* #	3.3±0.4*	3.9±0.4

* Highly significant difference *vs* controls $p< 0.01$.
#Significant difference *vs* group II $p<0.05$.
##Highly significant difference *vs* group II $p<0.01$.
CRP: C-reactive protein, IL-1ß: interleukin-1beta, IL-18: interleukin-18.

Table (5): Correlation matrix between the parameters studied in patient groups.

	CRP		IL 1B		IL-18	
	r	*P*	r	*P*	r	*P*
Creat.	.69	.001	.73	.001	.60	.001
Iron	-.79	.001	-.81	.001	-.67	.001
Ferritin	-.63	.001	-.70	.001	-53	.01
Hemoglobin	-.74	.001	-.87	.001	-.55	.01
Albumin	-.16	NS	-.28	.01	-.037	NS

4. Discussion:

CKD is a major health problem. Patients with ESRD are characterized by higher mortality rates than the general population. The majority of deaths in these patients are due to CVD[1].

CKD patients have an increased cardiovascular risk that cannot be explained completely by traditional cardiovascular risk factors. Inflammation plays a life-threatening pivotal role in the initiation and progression of atherosclerosis, and is considered a major non-traditional risk factor for accelerated carotid intima thickening and plaque formation in dialysis patients[24-25].

Protein-energy wasting (PEW) is common in patients with CKD and is associated with an increased death risk from CVD. However, while even minor renal dysfunction is an independent predictor of adverse cardiovascular prognosis, PEW becomes clinically manifest at an advanced stage, early before or during the dialytic stage[26].

The present study was designed to assess inflammatory and nutritional biomarkers in conjunction with echocardiographic assessment and carotid Duplex in CKD patients on HD or conservative treatment in a trial to clarify the role of inflammatory and nutritional biomarkers as risk factors for CV complications in these patients.

The results obtained revealed significant increase in systolic blood pressure in patients on HD (group I) and patients on conservative treatment (group II) versus the control group ($P< 0.01$) . Also a significant increase in diastolic blood pressure was found in group II versus the control group ($P< 0.05$). In agreement with these results Foley and Agarwal, [27] stated that the pathogenesis of hypertension in renal failure is complex and arises from the interaction of hemodynamic and neuroendocrine factors.

Regarding echocardiographic findings in patients of this current study, they showed that the mean ejection fraction (EF) and fractional shortening (FS) were significantly lower in both diseased groups in comparison to the control group ($P < 0.01$ and $P < 0.05$ respectively). This was in accordance with findings of Zeng and his colleagues[28] in their study of the value of B-type natriuretic peptide in diagnosing left ventricular dysfunction in dialysis dependent patients. The detection of systolic dysfunction appears particularly relevant in asymptomatic individuals where myocardial disease may progress despite compensatory mechanisms involving the autonomic system, neurohormones, and changes in cardiac function and structure. In this regard, the study of Zoccali *et al.* [29] was the first showing that LV systolic function measured by EF and FS predict incident CV events in a large population of asymptomatic ESRD patients. The prediction power of these indicators was largely independent of traditional and novel risk factors in ESRD such as CRP.

Although, there was a significant increase in left ventricular ESD in both diseased groups in comparison to control group ($P < 0.05$), yet there was no significant difference in EDD between patients and controls. This was in agreement with findings of Fathi et al. [30] who found no significant difference in EDD between patients and controls. Also Lisowska and Musial, [31] stated that heart failure is highly prevalent in ESRD patients. Upon starting dialysis, 37% of their patients had a previous episode of heart failure, thus doubling the risk of death, both systolic and/or

diastolic function may be impaired, 15% of patients on dialysis therapy may have systolic dysfunction.

In the current study, LV wall thickness (IVST and PWT), were hypertrophic in both patient groups compared to control group. This was in agreement with Fathi et al.[30] and Zaslavsky et al.[32] who reported increased posterior wall thickness in ESRD and HD patients.

Carotid duplex revealed a significant increase in IMT in both patient groups versus the control group (P<0.01). This finding was in accordance with findings of Masho and Shigematsu[33], who demonstrated that CKD patients are well recognized to have advanced arteriosclerosis with vascular medial calcification and with high risk of cardiovascular death. It is extremely important in order to prevent vascular calcification to adjust serum phosphorus, serum calcium and parathyroid function within the suitable range. In addition, hyperphosphatemia is becoming the powerful risk factor for patients' survival. In the current study, there was a significant increase in serum phosphorus in both patient groups versus the control group (P< 0.01). This is in agreement with the study done by Spasovski [34] who stated that the abnormalities in bone and mineral metabolism in CKD patients regarding hypocalcemia and hyperphosphatemia are associated with an increased risk of fractures, vascular calcifications and CVD.

The inflammatory markers CRP, IL-1ß and IL-18 exhibited significant elevation in both patient groups versus the control group (P < 0.01 for CRP , IL1ß and IL18). Additionally, their levels were significantly higher in HD patients compared to patients on conservative treatment (P < 0.01 for CRP, IL-1ß and IL-18). In agreement with these results , Jeznach-Steinhagen et al.,[35] reported that there is evidence that CKD patients are in a state of chronic inflammation with activation of C-reactive protein and proinflammatory cytokines and is associated with increased oxidative stress and endothelial dysfunction.

Atherosclerosis is the main cause of morbidity and mortality in patients with ESRD and there is consistent evidence that C-reactive protein (CRP) and proinflammatory cytokines such as interleukin-1ß (IL-1ß), interleukin-6 (IL-6) and tumor necrosis factor- α (TNF-α) are risk factors for atherosclerotic cardiovascular outcomes in these patients[10-11]. Tripepi and his colleagues [9] stressed that the severity of inflammation, as assessed by CRP and IL-6, adds significant prognostic information above and beyond traditional and nontraditional cardiovascular risk factors in dialysis patients. The impact of inflammation is more prominent in HD patients due to additional sources of inflammation[5], probably due to bioincompatible membranes that may activate the complement cascade[36], nonsterile dialysate with small amounts of endotoxin and the use of prosthetic arteriovenous grafts or transcutaneous catheters and processing the dialyser for reuse[37].

The prospective study of Blankenberg and his colleagues [38], demonstrated a strong and independent association between IL-18 level and future coronary events. In addition, Tomasz and his colleagues [39] stated that the contribution of IL-18, but not IL-6, to arteriosclerosis occurrence in CKD patients is independent from CRP or other factors involved in the general inflammatory process. Numerous reports indicate that patients with CKD have elevated serum levels of IL-18[40] , likely related to a greater percentage of active circulating monocytes, the main cellular source of this cytokine[41]. IL-18 may also accelerate the arterial injury in CKD through the induction of lymphocyte differentiation towards T-helper (Th-1) cells, the primary source of Interferon-γ (IFN-γ)[42-43].

In the present study, there was a significant decrease in the level of serum albumin, iron and ferrtin in both patient groups versus the control group and also in I group in comparison to group II (P<0.01 except for albumin between group I and II, P<0.05)). Amaral et al. [44] found no increase in CV mortality associated with low serum albumin level; however, they detected a 45% lower risk of hospitalization for CV events in patients with serum albumin more than 4.0g/dl.

Hypoalbuminemia is the most commonly used surrogate of PEW in dialysis patients and has a strong association with increased mortality[45] and morbidity[46]. Hypoalbuminemia is associated with development of de novo and recurrent cardiac failure in HD and peritoneal dialysis patients[47]. The use of serum prealbumin has been advocated as a better surrogate of nutritional status than albumin in dialysis patients[48]. A confounding factor is that serum albumin and prealbumin are also negative acute phase reactants and their serum levels are profoundly affected by the presence of an inflammatory response[26]. In dialysis patients, hypoalbuminemia could also be favored by the loss of amino acids and/or protein during renal replacement therapy[49]. Therefore, it is not clear whether the negative clinical outcome in advanced CKD patients associated with hypoalbuminemia is a reflection of nutrition or of the inflammatory response or both.

During the acute phase response, inflammatory cytokines such as IL-1ß and TNF-α increase the synthesis of both H and L subunits of ferritin[50]. Hence, serum ferritin can be elevated in inflammation. MICS may play a central role in poor clinical outcome including a high rate of mortality and hospitalization and diminished quality of life, it may also lead to hyperferritinemia and refractory anaemia including EPO hyporesponsiveness in these individuals.[22] It is not clear whether protein–energy malnutrition alone or

combined with inflammation in the form of MICS has a significant effect on serum ferritin in HD patients.[23] Kalantar-Zadeh and his colleagues found that low serum ferritin has a high specificity to detect iron deficiency in dialysis patients receiving EPO[51]. Indeed, inflammation may not have an effect on serum ferritin, unless there is enough iron stores in the body so that serum ferritin is somewhat increased. IL-1β induces ferritin gene expression by translational control of its mRNA; however, this inflammatory induction of ferritin synthesis is different from iron-dependent ferritin gene expression. They showed that this inflammatory regulation of ferritin requires the background presence of cellular iron[50]. In other words, without adequate iron stores, serum ferritin is low and does not correlate with inflammation, but with enough iron, serum ferritin is a function of both iron and inflammation. This important bench-research finding is consistent with our current and previous clinical and epidemiological findings that, in the setting of absolute iron deficiency, serum ferritin is almost always low. However, once the minimal required iron is available, ferritin regulation also becomes a function of non-iron-dependent factors such as inflammation[23].

The present work revealed a significant decrease in hemoglobin level in both diseased groups versus the control group and in HD patients in comparison to patients on conservative treatment ($P<0.01$). In agreement with these results, Iseki [52] stated that anemia develops during the early stages of CKD and is common in patients with ESRD. Anemia is an important cause of left ventricular hypertrophy and congestive heart failure. Treatment by erythropoietin is expected to improve quality of life, survival, and prevent the CKD progression.

A positive correlation was found between inflammatory factors (CRP,IL1ß and IL18) and urea and creatinine, while there was a negative correlation between nutritional factors(iron, ferritin and hemoglobin) and urea and creatinine. Also there was a negative correlation between inflammatory factors and nutritional factors which was in harmony with Kalantar-Zadeh and his colleagues who found simultaneous, significant correlations between serum ferritin and both markers of inflammation and iron status independent of each other.[23]

In our study, there was a positive correlation between CRP , IL1ß and IL 18 with both PWT&IVS with a positive correlation between CRP and ESD. Also there was a negative correlation between FS &EF and CRP &IL-18 and a positive correlation between them and nutritional markers (ferritin , hemoglobin &albumin). In agreement of our results, Erten and his colleagues proved that proinflammatory cytokines have an association with LVH in hemodialysis patients [53].

Conclusion:

CKD patients especially those on hemodialysis should be considered at high risk for developing CVD. The elevated levels of CRP and proinflammatory cytokines IL-1ß and IL-18 are associated with increased cardiovascular morbidity and may contribute to the deterioration of nutritional status in ESRD. Thus, it could be speculated that suppression of the vicious cycle of malnutrition, inflammation and atherosclerosis would improve survival in dialysis patients. As there is not yet any recognized, or even proposed, treatment for ESRD patients with chronic inflammation, it would be of obvious interest to study the long-term effect of various anti-inflammatory treatment strategies.

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5. References:

1. Himmelfarb J. (2005):Relevance of oxidative pathways in the pathophysiology of chronic kidney disease. Cardiol Clin., 23:319–330.
2. Stenvinkel P and Alvestrand A (2002): Inflammation in end-stage renal disease: sources, consequences and therapy Semin. Dial. 15: 329-337.
3. Stenvinkel P, Heimbürger O, Paultre F, *et al.*, (1999):Strong association between malnutrition, inflammation, and atherosclerosis in chronic renal failure.Kidney Int. 55(5):1899-1911.
4. Qureshi A, Alvestrand A, Divino-Filho J, *et al.* (2002):Inflammation, alnutrition, and cardiac disease as predictors of mortality in hemodialysis patients. J Am Soc Nephrol. 13:S28-36.
5. El-Shamy N; Abdel-Hadi A; Abu-Zihri N *et al.* (2003):Leptin: implication in malnutrition in uremic patients. Do inflammatory mediators interplay in this connection ? The Egyp. J. Int. Med. 15(3): 489-499,
6. Stenvinkel P, Heimburger O, Lindholm B, *et al.* (2000):Are there two types of malnutrition in chronic renal failure? Evidence for relationships between malnutrition, inflammation and atherosclerosis (MIA syndrome). Nephrol Dial Transplant 15(7):953-60.
7. Suliman ME, Stenvinkel P. (2009):Contribution of Inflammation to Vascular Disease in Chronic Kidney Disease Patients. Saudi J Kidney Dis Transpl 19:329-45.

8. Zoccali C; Mallamaci F and Tripepi G (2003):Inflammation and atherosclerosis in end stage renal disease. Blood Purif. 21:29-36.
9. Tripepi G; Mallamaci F and Zoccali C (2005):Inflammation markers, adhesion molecules, and all-cause and cardiovascular mortality in patients with ESRD: searching for the best risk marker by multivariate modeling. J. Am. Soc. Nephrol. 16 S83-S88.
10. Stenvinkel P; Barany P; Heimburger O *et al.* (2002):Mortality, malnutrition and atherosclerosis in ESRD: What is the role of interleukin 6? Kid. Int. 80:103-108.
11. Papagianni A; Kalovoulos M; Kirmizis D *et al.* (2003):Carotid atherosclerosis is associated with inflammation and endothelial cell adhesion molecules in chronic hemodialysis patients. Nephrol. Dial. Transplant. 18:113-119.
12. Honda H, Qureshi A, Heimburger O, *et al.* (2006): Serum Albumin, C-Reactive Protein, Interlukin6, and Fetuin A as predictors of malnutrition, cardiovascular disease, and mortality in patients with ESRD. Am J Kidney Dis 47(1):139-4811.
13. Maruyama Y, Nordfors L, Stenvinkel P, *et al.* (2005):Interleukin-1 gene cluster polymorphisms are associated with nutritional status and inflammation in patients with end-stage renal disease. Blood Purif. 23(5):384-93.
14. Whitman SC, Ravisankar P, Daugherty A. (2002):Interleukin-18 enhances atherosclerosis in apolipoprotein E(-/-) mice through release of interferon-gamma. Circ Res. 8;90(2):E34-8.
15. Stenvinkel P, Lindholm B and Heimburger O. (2004):Novel approaches in an integrated therapy of inflammatory-associated wasting in endstage renaldisease. Semin Dial; 17(6): 505-15.
16. Avesani CM, Carrero JJ, Axelsson J, *et al.* (2006) :Inflammation and wasting in chronic kidney disease: Partners in crime.Kidney Int (104):S8-13.
17. Bologa RM, Levine DM, Parker TS, *et al.* (1998):Interleukin-6 predicts hypoalbuminemia, hypocholesterolemia, and mortality in hemodialysis patients. Am J Kidney Dis 32(1):107-14.
18. Kaizu Y, Kimura M, Yoneyama T, *et al.* (1998): Interleukin-6 may mediate malnutrition in chronic hemodialysis patients. Am J Kidney Dis .,31(1):93-100.
19. Kaizu Y, Ohkawa S, Odamaki M, *et al.* (2003):Association between inflammatory mediators and muscle mass in long-term hemodialysis patients. Am J Kidney Dis 42(2):295-302.
20. Kalantar-Zadeh K, Kalantar-Zadeh K, Lee GH. (2006):The fascinating but deceptive ferritin: to measure it or not to measure it in chronic kidney disease? Clin J Am Soc Nephrol., 1:S9-S18.
21. Kalantar-Zadeh K, Ikizler TA, Block G and Avram MM. (2004):Malnutrition-inflammation complex syndrome in dialysis patients: Causes and consequences. Am J Kidney Dis 42(5): 864-81.
22. Kalantar-Zadeh K, McAllister C, Lehn R, *et al.* (2003):Effect of malnutrition–inflammation complex syndrome on erythropoietin hyporesponsiveness in maintenance hemodialysis patients. Am J Kidney Dis. 42(4):761–773 .
23. Kalantar-Zadeh K, Rodriguez RA and. Humphreys MH (2004):Association between serum ferritin and measures of inflammation,nutrition and iron in haemodialysis patients Nephrol Dial Transplant. 19: 141–149.
24. Sagun G, Kantarci G, Mesci B, *et al.* (2010):Frequency of cardiovascular risk factors and metabolic syndrome in patients with chronic kidney disease. Clin Med Res 8(3-4):135-41.
25. Wang AYM, Ho SSY, Liu EKH, *et al.* (2007): Differential association of traditional and non-traditional risk factors with carotid-intima-thickening and plaque in peritoneal dialysis patients. Am J Nephrol 27:458-65.
26. Bonanni A; Mannucci I; Verzola D *et al.* (2011): Protein-Energy Wasting and Mortality in Chronic Kidney Disease .Int. J. Environ. Res. Public Health, 8:1631-1654
27. Foley RN and Agarwal R. (2007): Hypertension is harmful to dialysis patients and should be controlled. Semin Dial. 20(6):518-22.
28. Zeng C, Wei T, Jin L, *et al.* (2006): Value of B-type natriuretic peptide in diagnosing left ventricular dysfunction in dialysis-dependent patients. Intern Med J. 36(9):552-557.
29. Zoccali,C., Benedetto,FA., Mallamaci,F., *et al.* (2004): Prognostic Value of Echocardiographic Indicators of Left Ventricular Systolic Function in Asymptomatic Dialysis Patients J Am Soc Nephrol 15:1029-1037.
30. Fathi R, Isbel N, Haluska B, *et al.* (2003): Correlates of subclinical left ventricular dysfunction in ESRD.Am J Kidney Dis. 41(5):1016-25.
31. Lisowska A and Musiał WJ. (2004):Heart failure in patients with chronic kidney disease. Rocz Akad Med Bialymst. 49:162-165.
32. Zaslavsky LM, Pinotti AFand Gross JL. (2005): Diastolic dysfunction and mortality in diabetic patients on hemodialysis: a 4.25-year controlled prospective study. Diabetes Complications 19(4):194-200.

33. Masho Y and Shigematsu T. (2007): Arteriosclerosis and vascular calcification in chronic kidney disease (CKD) patients.Clin Calcium. 17(3):354-9
34. Spasovski G. (2007): New aspects of treatment of renal bone disease in dialysis patients. Prilozi. 28(1):205-13.
35. Jeznach-Steinhagen A, Słotwiński R and Szczygieł B. (2007): Malnutrition, inflammation, atherosclerosis in hemodialysis patients.Rocz Panstw Zakl Hig. 58(1):83-8.
36. Memoli B, Postiglione L, Cianciaruso B, *et al.* (2000): Role of different dialysis membranes in the release of interleukin-6-soluble receptor in uremic patients.
Kidney Int. 58(1):417-24.
37. Tielemans C, Husson C, Schurmans T, *et al.* (1996): Effects of ultrapure and non-sterile dialysate on the inflammatory response during *in vitro* hemodialysis. Kidney Int. 49(1):236-43.
38. Blankenberg S; Luc G; Ducimetiere P *et al.* (2003):Interleukin-18 and the risk of coronary heart disease in European men: The prospective epidemiological study of myocardial infarction (PRIME).Circulation 108:2453-2454.
39. Tomasz P, Jakub K, Mariusz K, *et al.* (2009):IL-18 is involved in vascular injury in end-stage renal disease patients.Nephrol Dial Transplant 24: 589–596
40. Gangemi S, Mallamace A, Minciullo PL *et al.* (2002): Involvement of interleukin-18 in patients on maintenance haemodialysis. Am J Nephrol; 22: 417–421
41. Dinarello CA. (1999): IL-18: a TH1-inducing, proinflammatory cytokine and new member of the IL-1 family. J Allergy Clin Immunol; 103(1): 11–24
42. Descamps-Latscha B and Jungers P. (1996): New molecular aspects of chronic uraemia and dialysis-related immunocompetent cell activation. *Nephrol Dial Transplant*; 2(Suppl 11): 121–124
43. Sester U, Sester M, Hauk M *et al.* (2000): T-cell activation follows Th1 rather than Th2 pattern in haemodialysis patients. Nephrol Dial Transplant; 15: 1217–1223
44. Amaral S, Hwang W, Fivush B, *et al.* (2008):Serum albumin level and risk for mortality and hospitalization in adolescents on hemodialysis. Clin J Am Soc Nephrol 3: 759-767.
45. Kalantar-Zadeh K, Regidor DL, McAllister CJ, *et al.* (2005): Time-dependent associations between iron and mortality in hemodialysis patients. J Am Soc Nephrol16:3070 –3080.
46. Ikizler TA, Wingard RL, Harvell J, *et al.* (1999): Association of morbidity with markers of nutrition and inflammation in chronic hemodialysis patients: A prospective study.Kidney Int. 55:1945–1951.
47. Foley RN, Murray AM, Li S, *et al.* (2005): Chronic kidney disease and the risk for cardiovascular disease, renal replacement, and death in the United States Medicare population, 1998 to 1999.J Am Soc Nephrol. 16(2):489-95.
48. Chertow GM, Goldstein-Fuchs DJ, Lazarus JM, *et al.* (2005): Prealbumin, mortality, and cause-specific hospitalization in hemodialysis patients. Kidney Int. 68: 2794–2800.
49. Gil HW, Yang JO, Lee EY, *et al.* (2007) :The effect of dialysis membrane flux on amino acid loss in haemodialysis patients. J. Korean Med. Sci. 22:598–603.
50. Rogers JT, Bridges KR, Durmowicz GP, *et al.* (1990): Munro HN. Translational control during the acute phase response. Ferritin synthesis in response to interleukin-1. J Biol Chem ., 265: 14572–14578
51. Kalantar-Zadeh K, Hoffken B, Wunsch H, *et al.* (1995):Diagnosis of iron deficiency anemia in renal failure patients during the post-erythropoietin era. Am J Kidney Dis 26: 292–299
52. Iseki K and Kohagura K. (2007): Anemia as a risk factor for chronic kidney disease.Kidney Int Suppl.(107):S4-9.
53. Erten Y, Tulmac M, Derici U, et al., (2005) An Association between inflammatory state and left ventricular hypertrophy in hemodialysis patients. Ren Fail 27(5):581-589.

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