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BRIEF ARTICLE

Inflammatory status in chronic renal failure: The role of homocysteinemia and pro-inflammatory cytokines

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Abstract

AIM: To evaluate determinants of inflammatory markers in chronic renal failure patients according to the level of glomerular filtration rate.

METHODS: One hundred fifty four patients (Age: 44 ± 06 years; male/female: 66/88) with chronic renal failure (CRF) were divided into 6 groups according to the National Kidney Foundation (NKF) classification. They included 28 primary stage renal failure patients (CRF 1), 28 moderate stage renal failure patients (CRF 2), 28 severe stage renal failure patients (CRF 3), 18 end-stage renal failure patients (CRF 4), 40 hemodialysis (HD) patients, and 12 peritoneal dialysis (PD) patients. Tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6) and C-reactive protein (CRP) were analyzed by immunosorbent assay kit (ELISA) (Cayman Chemical's ACETM EIA kit). Immunoassay methods were used for total homocysteine (tHcy) (fluorescence polarization immunoanalysis HPLC, PerkinEmer 200 series), transferrin (MININEPHTM human transferin kit: ZK070.R), ferritin (*ADVIA Centaur*) and fibrinogen analysis (ACL 200). Differences between groups were performed using SPSS 20.0 and data are expressed as the mean ± SD.

RESULTS: Results showed that in comparison with CRF 1 group and other groups, TNF-α and IL-6 levels were respectively more elevated in HD (16.38 ± 5.52 pg/mL *vs* 0.39 ± 0.03 pg/mL, 11.05 ± 3.59 pg/mL *vs* 8.20 ± 0.22 pg/mL, $P < 0.001$) and PD (14.04 ± 3.40 pg/mL *vs* 0.39 ± 0.03 pg/mL, 10.15 ± 1.66 pg/mL *vs* 8.20 ± 0.22 pg/mL, $P < 0.001$). IL-1β levels were increased in HD (9.63 ± 3.50 pg/mL *vs* 3.24 ± 0.10 pg/mL, $P < 0.001$) and CRF 4 (7.76 ± 0.66 pg/mL *vs* 3.24 ± 0.10 pg/mL, $P < 0.001$) patients than in CRF 1 and in the other groups. Plasma tHcy levels were higher in HD (32.27 ± 12.08 μmol/L) and PD (28.37 ± 4.98 μmol/L) patients compared to the other groups of CRF ($P < 0.001$). The serum CRP level was significantly increased in HD (18.17 ± 6.38 mg/L) and PD (17.97 ± 4.85 mg/L) patients compared to the other groups of CRF patients ($P < 0.001$). The plasma fibrinogen level was more elevated in HD (6.86 ± 1.06 g/L) and CRF 4 (6.05 ± 0.57 g/L) than in the other groups ($P < 0.001$). Furthermore; the ferritin level was higher in HD (169.90 ± 62.16 ng/mL) and PD (90.08 ± 22.09 ng/mL) patients compared to the other groups of CRF ($P < 0.001$). The serum transferrin value was significantly decreased especially in PD (1.78 ± 0.21 g/L) compared to the other groups ($P < 0.001$). We found a negative correlation

between glomerular filtration rate (GFR), TNF-α levels (r = -0.75, P < 0.001), and tHcy levels (r = -0.68, P < 0.001). We observed a positive correlation between GFR and transferrin levels (r = 0.60, P < 0.001).

CONCLUSION: CRF was associated with elevated inflammatory markers. The inflammation was observed at the severe stage of CRF and increases with progression of renal failure.

Key words: Chronic renal failure; Inflammation; Pro-inflammatory cytokines; Total homocysteine; Glomerular filtration rate

Core tip: Chronic inflammation is highly prevalent in patients with chronic renal failure (CRF). The aim of this study was to evaluate determinants of inflammatory markers in CRF patients. One hundred fifty four CRF patients were divided into 6 groups according to the National Kidney Foundation classification. Tumor necrosis factor-α, interleukin-1β, interleukin-6 values were elevated in hemodialysis (HD) and peritoneal dialysis (PD) patients compared to the other groups of CRF patients. Compared to others stages of CRF patients, total homocysteine, C-reactive protein, ferritin and fibrinogen were increased in HD and PD patients while; transferrin was decreased only in PD. CRF was associated with an enhanced inflammatory reaction existing already at primary stages. This situation was aggravated by the progression disease and dialysis procedure.

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INTRODUCTION

Chronic inflammation is prevalent in patients with chronic renal failure (CRF)[1]. Numerous studies have demonstrated that chronic inflammation may contribute to the morbidity and mortality among dialysis patients[2]. Indeed, deterioration of renal function in uremia increases risk to infection and various abnormalities of the immune system[3]. In addition, the repeated dialysis treatments in patients lead to leucocyte activation and consequently the production of cytokines[4].

The upregulation and presence of cytokines such as; tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and IL-6, in the blood contribute to chronic inflammation[5].

In the uremic state, the inflammatory response is characterized by an obvious presence of pro-inflammatory cytokines. By the following, this presence implies activation of the acute phase proteins such as C-Reactive Protein (CRP) and fibrinogen[6,7].

In dialysis patients, in particularity hemodialysis (HD), the repeated acute phase activation causes chronic inflammation, which produces in the long-term variability of complications, such as malnutrition and cardiovascular disease (CVD)[8].

Hyperhomocysteinemia is overrepresented in patients with end stage renal failure, raising the risk of cardiovascular disease (CV). The prevalence of hyperhomocysteinemia in patients with moderate CRF is less well known, although it has been demonstrated that homocysteine levels are closely related to plasma creatinin concentration[9]. Moreover, it has been showed that glomerular filtration rate (GFR) is a strong determinant of plasma homocysteine and cysteine concentrations in end stage renal disease patients[10].

Moreover, a high level of total plasma homocysteine (tHcy) appears as an independent risk factor for CVD in the general population; however, conflicting findings are reported regarding the association between plasma tHcy level and the prevalence of CVD in patients with CRF. Some studies showed poor outcomes in HD patients with hyperhomocysteinemia[11]. The work of Ducloux *et al*[12] reported that a high tHcy level is associated with a higher risk of all-causes mortality in dialysis patients.

In this study, we aim to evaluate the inflammation indices in patient with different stage of CRF. We analyze the pro-inflammatory cytokines, such as TNF-α, IL-β, IL-6 and their relationship with the other markers of inflammation. We also, evaluate the role of homocysteinemia in CRF inflammation.

MATERIALS AND METHODS

The study was carried out on 154 CRF patients. Patients were divided into 6 groups according to the classification of the National Kidney Foundation (NKF)[13]. They included 28 primary stage renal failure patients (CRF 1), 28 moderate stage renal failure patients (CRF 2), 28 severe stage renal failure patients (CRF 3), 18 end-stage renal failure patients (CRF 4), 40 hemodialysis (HD) patients, and 12 peritoneal dialysis (PD) patients (Table 1).

We excluded from our study patients which present clinical signs of infection (hepatitis B, hepatitis C), malignancy, active immunological diseases, immunosuppressive or immunomodulatory and anti-inflammatory drugs (*i.e.*, conditions that conceal cytokine release). We also excluded patients with diabetes and nephrotic syndrome, because diabetes could induce an inflammatory response. None of the patients were taking lipid-lowering drugs or antioxidant supplements. Patients take hypotensives drugs, calcium, vitamin D and erythropoietin.

The etiology of CRF in our study included; hypertension (66%), cystic kidney disease (14%), glomerulonephritis (6%), prostatic obstruction (3%), pulmonary tuberculosis treatment (1%) and (10%) were unknown.

We calculate the creatinin clearance from the serum

Table 1 Clinical and biochemical characteristics of patients

	CRF 1 (*n* = 28)	CRF 2 (*n* = 28)	CRF 3 (*n* = 28)	CRF 4 (*n* = 18)	HD (*n* = 40)	PD (*n* = 12)
Age (yr)	37 ± 13	55 ± 11	45 ± 15	46 ± 14	42 ± 11	39 ± 15
Weight (kg)	66 ± 12.74	67 ± 16.12	67 ± 13.96	61 ± 13.87	56.44 ± 11.37	68.22 ± 10.55
BMI (kg/m^2)	24.07 ± 7.16	25.35 ± 5.63	24.91 ± 3.54	23.63 ± 4.01	23.15 ± 3.02	25.43 ± 3.28
Sex ratio (M/F)	10/18	11/17	10/18	7/11	22/18	6/6
GFR (mL/min)	86.25 ± 23.87	46 ± 6.95	20.31 ± 5.10	10.75 ± 2.56	-	-
Dialysis duration (mo)	-	-	-	-	14-109	05-49
Glucose (g/L)	0.85 ± 0.12	0.85 ± 0.13	0.89 ± 0.10	0.83 ± 0.13	0.81 ± 0.16	0.87 ± 0.14
Urea (g/L)	0.34 ± 0.15	0.53 ± 0.20	1.05 ± 0.34	1.67 ± 0.82	1.32 ± 0.36	1.04 ± 0.38
Creatinin (mg/L)	10.30 ± 2.89	18.54 ± 5.26	41.75 ± 11.46	70.03 ± 26.15	102.12 ± 29.37	69.36 ± 36.95
Total proteins (g/L)	75.17 ± 5.21	74.29 ± 7.52	71.16 ± 11.15	70.86 ± 10.52	69.92 ± 9.60	67.04 ± 7.36
Albumin (g/L)	46.49 ± 3.48	44.43 ± 2.41	43.93 ± 3.86	42.75 ± 7.33	41.40 ± 4.48	40.32 ± 6.66
Cholesterol (g/L)	1.68 ± 0.33	1.89 ± 0.29	1.73 ± 0.32	1.74 ± 0.45	2.17 ± 1.58	1.95 ± 0.56
HDL-cholesterol (g/L)	0.53 ± 0.10	0.48 ± 0.09	0.50 ± 0.10	0.44 ± 0.11	0.36 ± 0.11	0.42 ± 0.07
LDL-cholesterol (g/L)	0.93 ± 0.33	1.13 ± 0.22	0.95 ± 0.93	0.94 ± 0.41	1.14 ± 0.51	1.21 ± 0.45
Triglycerides (g/L)	1.17 ± 0.53	1.39 ± 0.55	1.47 ± 0.74	1.30 ± 0.55	1.64 ± 0.90	1.75 ± 0.89
Aspartateaminotransferase (ASAT, TGO) (U/L)	25.26 ± 12.10	28.32 ± 12.78	22.49 ± 5.59	23.25 ± 11.03	17.49 ± 7.34	23.50 ± 11.50
Alanine aminotransferase (ALAT, TGP) (U/L)	21.74 ± 13.55	22.71 ± 15.80	12.88 ± 7.69	16.00 ± 7.24	16.31 ± 9.70	21.00 ± 17.26
Alkaline phosphatase (U/L)	68.42 ± 30.74	99.69 ± 47.16	92.88 ± 31.20	98.00 ± 29.55	88.73 ± 33.23	96.25 ± 32.63
Gamma-glutamyl transferase (U/L)	15 ± 7.11	26.94 ± 34.53	22.27 ± 12.19	28.17 ± 16.90	32.18 ± 20.40	26.00 ± 17.10
Hemoglobin (g/dL)	13.01 ± 1.36	12.28 ± 1.31	11.46 ± 1.53	8.71 ± 1.37	9.55 ± 1.86	10.38 ± 2.44

BMI: Body mass index (weight kg/height m^2); GFR: Glomerular filtration rate; HD: Hemodialysis; PD: Peritoneal dialysis; M: Male; F: Female; HDL: High density lipoprotein; LDL: Low density lipoprotein. Data are spoken in mean ± SD.

creatinin and through the following estimation formula: the Cockroft and Gault[14] formula [GFR ¼ (140 - age) BW × 1.23/creatinin]. In women, this value was multiplied by 0.85. Hemodialysis patients were on standard bicarbonate using Polysulfone membrane. Patients were dialyzed since 12 to 60 mo, three times a week, and each session lasting 4 h. Peritoneal dialysis patients were in dialysis since 3 to 48 mo, using a standard procedure (four exchanges: Three isotonic 1.36% glucose solutions, then a hypertonic one at 3.86% glucose). We note that the patients' nutrition contains small amounts of protein and phosphate.

All patients were treated at the Nephrology ward of the University Hospital of Oran. The purpose of this study was explained to the subjects and the investigation was carried out with their consent. The experimental protocol was approved by the Committee for Research on Human Subjects of Oran.

Assays

In all patients, blood samples were drawn after 12-h overnight fast from antecubital venipuncture in CRF and PD patients and by the dialysis fistule in HD patients. We used vacutainer tubes with different anticoagulants during the blood samples. We give the features of those tubes. Tubes containing the lithium heparin for biochemical experiments, those containing ethylene diamine tetraacetic acid (EDTA) 8% for the hematology detection and tubes containing sodium citrate 3.2% (as an anticoagulant) for the hemostasis coagulation tests, and dry tube for an appropriate hormonology and an immunology analysis. We collected plasma and serum according to the technique low speed centrifugation at 3000 × *g* at 4 ℃, for 15 min. The fresh plasma was collected and analyzed on the same day. The serum was removed, aliquoted and stored at -20 ℃. We used this serum for cytokines, CRP, Ferritin and transferrin analysis.

Markers of inflammation analysis

For the aim of our study, we determined the following markers; TNF-α, IL-1β and IL-6 in duplicate samples with a commercial enzyme-linked immunosorbent assay kit (ELISA) (Cayman Chemical's ACE™ EIA kit) with a range of 0-250 pg/mL. The lower limit of detection is 3.9 pg/mL for TNF-α and IL-1β, 7.8 pg/mL for IL-6.

We measured the plasma tHcy according to the fluorescence polarization immunoanalysis (HPLC, PerkinElmer 200 series). The both forms of plasma homocysteine, reduced and oxidized (*i.e.*, homocysteine-cysteine mixed disulfide, and protein-bound homocysteine mixed disulfide) were determined in this analysis. These forms are collectively referred to as the total plasma homocysteine. A reference range for plasma tHcy was compressed between 5.0-14 μmol/L.

For the CRP marker, we measured it in duplicate samples with an immunometric assay kit (ELISA) (Cayman Chemical's ACE™ EIA kit) with a range of 0-3000 pg/mL and with a limit of detection of approximately 50 pg/mL. For the fibrinogen marker, we measured it across an automatic analyzer (ACL 200).

Finally, we measured the ferritin marker through an immunoanalyzer (ADVIA Centaur), while we measured the transferrin across a nephelometric immunoassay (MININEPH™ human transferin kit: ZK070.R).

Table 2 Inflammation markers, total homocysteine and cytokines in chronic renal failure patients

	CRF 1	CRF 2	CRF 3	CRF 4	HD	PD	*P*
TNF-α (pg/mL)	0.39 ± 0.03	3.81 ± 1.52	7.80 ± 2.76	10.04 ± 1.61	16.38 ± 5.52	14.04 ± 3.40	0.001
IL-1β (pg/mL)	3.24 ± 0.10	3.57 ± 0.47	6.69 ± 1.02	7.76 ± 0.66	9.63 ± 3.50	6.51 ± 1.37	0.001
IL-6 (pg/mL)	8.20 ± 0.22	8.79 ± 1.04	8.88 ± 0.71	9.66 ± 0.56	11.05 ± 3.59	10.15 ± 1.66	0.001
tHcy (μmol/L)	8.05 ± 2.43	11.95 ± 5.26	20.16 ± 2.62	25.34 ± 3.93	32.27 ± 12.08	28.37 ± 4.98	0.001
CRP (mg/L)	3.29 ± 2.33	5.54 ± 2.16	8.18 ± 3.33	13.5 ± 4.28	18.17 ± 6.38	17.97 ± 4.85	0.001
Fib (g/L)	3.58 ± 0.82	4.51 ± 0.47	5.80 ± 1.21	6.05 ± 0.57	6.86 ± 1.06	5.91 ± 0.59	0.001
Ferritin (ng/mL)	47.66 ± 25.24	51.12 ± 34.57	74.22 ± 43.18	83.83 ± 44.99	169.90 ± 62.16	90.08 ± 22.09	0.001
Transferrin (g/L)	2.73 ± 0.47	2.41 ± 0.23	2.29 ± 0.37	2.03 ± 0.49	1.86 ± 0.39	1.78 ± 0.21	0.001

Data are presented as the mean ± SD. Statistically significant differences between all the groups ($P < 0.001$). TNF-α: Tumor necrosis factor-α; IL-1β: Interleukin-1β; IL-6: Interleukin-6; tHcy: Total homocysteine; CRP: C-reactive protein; Fib: Fibrinogen; HD: Hemodialysis; PD: Peritoneal dialysis.

Statistical analysis

Statistical analysis is performed using SPSS 20.0 (IBM SPSS statistics. Version 20). Data are expressed as the mean ± SD. The distribution of variables is compared by the c2 analysis. The difference between the arithmetical averages is assessed by ANOVA, which is adjusted for multiple comparisons. Depending on the normality of distribution of variables, the comparisons between groups are performed using way analysis of variance (ANOVA) or the Mann-Whitney *U*-test when results are non-parametrically distributed. The coefficient of variation is defined as the standard deviation percentage of the mean. In order to determine the correlation between inflammatory markers and GFR, we propose to calculate the Spearman coefficients (*r*). Significance tests are two-sided, and values $P < 0.05$ are considered statistically significant.

RESULTS

The result of our experiments on the pro-inflammatory cytokines; tHcy, CRP and fibrinogen were reported on Table 2. TNF-α was 4.2-fold higher in HD (16.38 ± 5.52 pg/mL), 3.6-fold higher in PD (14.04 ± 3.40 pg/mL) and 2.5-fold higher in CRF 4 (10.04 ± 1.61 pg/mL) than in CRF 1 patients (0.39 ± 0.03 pg/mL) ($P < 0.001$). IL-6 was higher 1.3-fold in HD (11.05 ± 3.59 pg/mL), 1.2-fold in PD (10.15 ± 1.66 pg/mL) and 1-fold in CRF 4 (9.66 ± 0.56 pg/mL) than in CRF 1 (8.20 ± 0.22 pg/mL) patients ($P < 0.001$). IL-1β was 2.9-fold higher in HD (9.63 ± 3.50 pg/mL), 2.4-fold higher in CRF 4 (7.76 ± 0.66 pg/mL) and 2-fold higher in PD (6.51 ± 1.37 pg/mL) than in CRF 1 patients (3.24 ± 0.10 pg/mL) ($P < 0.001$). We observed an increase in the levels of TNF-α, IL-1β and IL-6. We noted that the markers TNF-α, IL-1β and IL-6 levels were increased at stage 3 and stage 4 of CRF. It is important to note that the levels of the latter markers were significantly higher in HD and in PD patients than in the other stages of CRF patients ($P < 0.001$).

In addition, our results showed high values of plasma tHcy. The tHcy levels were 4-fold higher in HD (32.27 ± 12.08 μmol/L), 3.5-fold higher in PD (28.37 ± 4.98 μmol/L) and 3-fold higher in CRF 4 (25.34 ± 3.93 μmol/L) than in CRF 1 (8.05 ± 2.43 μmol/L) patients ($P < 0.001$). The values were increased at stage 3 and stage 4 of CRF. Also, we noted that homocysteinemia was significantly higher in HD and in PD patients than in the other stages of CRF patients ($P < 0.001$).

We also observed that CRP and plasma fibrinogen concentrations were increased with cytokines and tHcy. The CRP was 5.5-fold higher in HD (18.17 ± 6.38 mg/L), 5.4 higher in PD (17.97 ± 4.85 mg/L) and 4-fold higher in CRF 4 (13.5 ± 4.28 mg/L) than in the CRF 1 patients (3.29 ± 2.33 mg/L) ($P < 0.001$). The CRP levels were increased at stage 3 and stage 4 of CRF. Also, it is important to note that the levels of CRP was significantly higher in HD and PD patients than in the other stage of CRF patients ($P < 0.001$). The plasma fibrinogen levels were significantly higher in HD patients than in the other groups of CRF ($P < 0.001$). Indeed, the fibrinogen levels are 2-fold higher in HD (6.86 ± 1.06 g/L), 1.6-fold higher in PD (5.91 ± 0.59 g/L) and CRF 4 (6.05 ± 0.57 g/L) than in CRF 1 (3.58 ± 0.82 g/L) patients. Furthermore, the fibrinogen level was 1-fold more elevated in HD patients than in PD patients ($P < 0.001$).

Ours results on the ferritin and the transferrin were also reported on Table 2. The ferritin level was 4-fold higher in HD (169.90 ± 62.16 ng/mL), 3.5-fold higher in PD (90.08 ± 22.09 ng/mL) and 3-fold higher in CRF 4 (83.83 ± 44.99 ng/mL) than in CRF 1 (47.66 ± 25.24 ng/mL) patients. This level of the ferritin was also 1.5-fold higher in HD than in PD patients. We observed a highly significant increase in the ferritin level in HD ($P < 0.001$) and PD ($P < 0.001$) patients compared to the other groups of CRF.

The transferrin level was decreased in HD and PD patients than in the other stages of CRF patients ($P < 0.001$). Transferrin concentrations were diminished by -68% in HD (1.86 ± 0.39 g/L) patients and by -65% in PD patients (1.78 ± 0.21 g/L) than in CRF1 patients (2.73 ± 0.47 g/L).

Correlation analysis

The result of the correlation analysis was reported in Table 3. We found a strong positive correlation between levels of TNF-α and, IL-1β, IL-6, tHcy, CRP, fibrinogen and ferritin in CRF patients. However, we found a negative correlation between TNF-α and transferrin amounts. On the other hand, we found a negative correlation between the GFR and, pro-inflammatory cytokines, tHcy,

Table 3 Correlation among inflammation markers, total homocysteine and cytokines in chronic renal failure patients and glomerular filtration rate in uremic patients

	TNF-α	GFR
TNF-α	-	-0.75 $P < 0.001$
IL-1β	0.75 $P < 0.001$	-0.64 $P < 0.001$
IL-6	0.39 $P < 0.001$	-0.36 $P < 0.001$
tHcy	0.73 $P < 0.001$	-0.68 $P < 0.001$
CRP	0.72 $P < 0.001$	-0.71 $P < 0.001$
Fib	0.66 $P < 0.001$	-0.71 $P < 0.001$
Ferritin	-	-0.48 $P < 0.001$
Transferrin	-	0.60 $P < 0.001$

TNF-α: Tumor necrosis factor-α; IL-1β: Interleukin-1β; IL-6: Interleukin-6; tHcy: Total homocysteine; CRP: C-reactive Protein; Fib: Fibrinogen; GFR: Glomerular filtration rate.

CRP, fibrinogen and ferritin. Moreover, we observed a positive correlation between the GFR and the transferrin.

DISCUSSION

In this study, we analyze the pro-inflammatory cytokines in CRF: TNF-α, IL-1β, IL-6 and their relationship with the other markers of inflammation. We found that the cytokine levels were higher in HD patients than in PD patients and in the other stages of CRF patients. Cytokines concentrations were also elevated in PD, CRF 3 and CRF 4 patients. Ours results were in accordance with previous findings[15]. As previously described, we suggested that the cytokine increase was caused either by the endotoxines-stream in uremic and HD patients, or by the non-endotoxemic cytokine that activate receptors[16]. In our study, TNF-α concentrations were positively correlated with both IL-1β and IL-6.

Our results showed that the CRP levels were more elevated in HD patients compared to CRF and PD groups. Both of CRP and IL-6 levels were elevated in HD patients. These results were in accordance with others studies[17-19]. In addition, many related works have reported the existence of an association between the concentration of CRP and both vascular inflammation[20] and atherosclerosis in CRF[21].

For the fibrinogen levels, we observed a significant increase in HD, PD and all CRF groups. We found also a strong positive correlation between TNF-α and fibrinogen concentrations. Our result was in accordance with the study that describes the effect of an inflammatory reaction, which increases the fibrinogen level[22].

In our experiments, we found that the plasma tHcy was more increased in HD patients compared to PD patients and to the other stages of CRF patients. tHcy was also increased in PD, CRF 3 and CRF 4 patients. In agreement with others studies, hyperhomocysteinemia was present in the majority of CRF patients; they have concentration of tHcy elevated 3- to 4-fold above normal[23]. In addition, tHcy could be a principal candidate for endothelial dysfunction in patients with CRF, which a positive association between a high tHcy level and CVD in the general population[24] and in HD patients[11,25]. Otherwise, the role of homocysteine in atherosclerosis is also controversial, although some authors have associated the hyperhomocysteinemia with this disease[26].

Malnutrition and inflammation have been simultaneous reported in the CRF patients. We observed significant associations between concentrations of CRP, transferrin and ferritin. In fact, transferrin is the negative acute-phase protein. We observed that patients in end stage renal disease (CRF 4), and those on HD and PD presented low level of transferrin. This result explains the presence of an ongoing inflammatory state.

In the case of CRP, we believe that the inflammation leads to the malnutrition. It is worth nothing that our obtained result was in accordance with the results showed in other study[27]. In addition, authors[28,29] have also showed that the pro-inflammatory cytokines can lead to anorexia and erythropoiesis impairment. It has demonstrated[30] that TNF-α and IL-6 cytokines were attached to the muscle. They cause the catabolism and the abolition the protein synthesis (the muscle protein). Such effects[31] (*i.e.*, the catabolism and the abolition the protein synthesis) straight stimulate proteolysis either by the cytokines or by the insulin resistance. The latter is responsible of the anorexia development.

Compared to other study[32], the advanced stages of CRF lead on one hand, to an increase of cytokines production, and on another hand to a decrease of the renal clearance. We noted that the levels of pro-inflammatory cytokine (TNF-α, IL-1β, IL-6) and tHcy increase in patients with end-stage renal failure and dialysis patients (in both HD and PD patients). Also, high levels of pro-inflammatory cytokine (TNF-α, IL-1β, IL-6) and tHcy step up the cardiovascular risk. They are inversely correlated with GFR, while; the transferrin was positively correlated with the latter. Our results were also in accordance with others works[6,33,34].

In conclusion, we show that the increase of inflammation markers already exist at stage 3 and stage 4 of CRF leading to an inflammatory reaction. These abnormalities can lead to serious complications of CRF, such as; CVD, atherosclerosis, malnutrition and the anemia. Further, the disease progression and the dialysis treatment may also aggravate the markers evaluation.

COMMENTS

Background

Inflammatory complications are the leading cause of cardiovascular disease and malnutrition in chronic renal failure patients. Progression of this disease is associated with an increase synthesis of pro-inflammatory cytokines and homocysteine, a crucial event in evolution of chronic renal failure.

Research frontiers

Pro-inflammatory cytokines are the principal mediators of the inflammatory reaction they are produced mainly by immune cells. Homocysteine is an intermediate product of methionine metabolism. Special focus was laid on those latter and theirs role of the evolution of chronic renal failure.

Innovations and breakthroughs

Recent studies have identified the tumor necrosis factor-α, interleukin-1β, and interleukin-6 as the principal cytokines that contribute to the inflammation.

Determination of the levels of these cytokines, homocysteine and their association during evolution of chronic renal failure has not been previously reported in chronic renal failure patients at different sage of disease.

Applications

By understanding how the pro-inflammatory cytokines and homocysteine are induced and by reducing theirs synthesis, this study may represent a future strategy for therapeutic and nutritional interventions in the prevention of malnutrition and cardiovascular disease in patients with chronic renal failure.

Peer review

This interesting study demonstrates that the levels of pro-inflammatory cytokines and homocysteine increases with evolution of renal failure. Pro-inflammatory cytokines and homocysteine are involved in the pathogenesis of inflammation in chronic renal failure and theirs association with the immune system may result in a potential therapeutic target.

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