Level of inflammatory factors in chronic hemodialysis patients with and without cardiovascular disease

Shahram Taheri, Azar Baradaran[1], Mahnaz Aliakbarian[1], Mojgan Mortazavi

Department of Internal Medicine, Isfahan Kidney Diseases Research Center, Isfahan University of Medical Sciences, [1]Department of Pathology, Isfahan University of Medical Sciences, Isfahan, Iran

Background: Considering the existence of controversies about the predictive value of inflammatory markers for cardiovascular disease (CVD), we aimed to compare the level of high-sensitivity C-reactive protein (hs-CRP) and interlukin-6 (IL-6) level in chronic hemodialysis (HD) patients with and without CVD. **Materials and Methods:** In this historical cohort study, HD patients with and without CVD disease were enrolled. The presence of CVD risk factors, level of inflammatory factors including IL-6 and hs-CRP as well as lipid levels, fasting blood sugar, and other biochemical factors were compared in two studied groups. **Results:** During the study, eighty HD patients with ($n = 40$) and without ($n = 40$) CVD were enrolled. Diabetes was more prevalent among HD patients with CVD than those without CVD ($P < 0.05$). The level of IL-6 and hs-CRP were not different in two studied groups ($P > 0.05$). Univariate analysis of variance test indicated that there was not any significant relationship between hs-CRP and CVD ($P > 0.05$). **Conclusion:** The findings indicated that the level of inflammatory factors including hs-CRP and IL-6 are not significantly different in HD patients with and without CVD. However, for obtaining more definite conclusion in this field and evaluation their predicting role in this field, it is recommended to study other novel inflammatory markers as well as the additive effect of the inflammatory factors with traditional ones in larger sample size and longer follow-up.

Key words: Cardiovascular disease, cardiovascular risk factor, high sensitivity C-reactive protein, interleukin-6

INTRODUCTION

Cardiovascular disease (CVD) is the main cause of morbidity and mortality in patients with end-stage renal disease (ESRD).[1] It is reported that the rate of mortality in chronic hemodialysis (HD) is 7–8 times greater than general population, and 40%–45% of all causes of mortality are related to CVD.[2,3] According to the recommendation of the American Heart Association, ESRD is considered as coronary heart disease risk equivalent.[4]

Although different traditional cardiovascular risk factors including Framingham Risk Score charts have been developed and evaluated for predicting CVD risk in chronic HD patients, epidemiological studies relieved that these risk factors could not be sufficiently used as risk predictors of CVD. Hence, usefulness of other nontraditional risk factors involved in the pathogenesis of uremic CVD has been investigated in some studies.[5-7]

Evidence suggested that many pathophysiologic mechanisms such as inflammation, oxidative stress, endothelial dysfunction, and vascular calcification could explain the process of CVD in patients with ESRD.[8]

The role of chronic inflammation in related adverse cardiovascular outcomes and high mortality rate of ESRD have been demonstrated in many studies, and several pathogenetic mechanisms have been introduced in this regard.[9-11] There is consistent evidence that

Address for correspondence: Dr. Mahnaz Aliakbarian, Department of Pathology, Al-Zahra University Hospital, Sofeh Avenue, P. O. Box: 8174675731, Isfahan, Iran. E-mail: mahnaz.aliakbarian@gmail.com

pro-inflammatory cytokines serve as an important modulator in the genesis of both vascular and nutritional risk factors in ESRD.[12] In addition, there is a significant association between chronic inflammation and endothelial dysfunction and increased oxidative stress in this group of patients.[13]

Although there is not any definite definition for inflammation in patients with ESRD, according to the National Kidney Foundation K/DOQI guidelines, increased the level of C-reactive protein (CRP) could be used as an inflammatory marker.[14]

On the other hand, pro-inflammatory cytokines such as interlukin-6 (IL-6) is a primary determinant of hepatic production of CRP.[15] These inflammatory biomarkers are highly prevalent in HD patients, and some studies reported that they could be used as independent predictors of CVD.[16-18] There are still controversies in this regard, and some studies did not confirm the predictive role of mentioned factors.[19,20]

It is well established that expression of these factors affected by different factors such as genetic and ethnic ones.[21]

Considering that identification the role of these inflammatory factors in this field and their suppression would improve the survival of patients with chronic HD and the existence of controversies about the predictive value of these markers for CVD, we aimed to compare the level of high-sensitivity CRP (hs-CRP) and IL-6 level in chronic HD patients with and without CVD.

MATERIALS AND METHODS

In this historical cohort study, eighty patients, forty in each with and without cardiovascular events, on chronic HD in two referral hospital HD centers were enrolled. The study was conducted from March 2013 to March 2014 in Isfahan city.

The protocol of study was approved by the Regional Ethics Committee of Isfahan University of Medical Sciences (research project number 392546).

HD patients with and without CVD were consequently selected according to following criteria. All selected patients were above 18-year-old, had been undergoing regular HD for more than 3 months and did not have acute illnesses, history of immunologic disease, and steroid therapy.

Patients who had history of coronary artery bypass surgery, percutaneous transluminal coronary angioplasty, and coronary or cerebral or peripheral artery occlusive disease,

and those who used medication for CVD were classified in group with CVD.

Written informed consent was obtained from the selected patients after describing the methods and aims of the study for all participants.

The presence of traditional CVD risk factors in two groups were recorded.[22] Lipids level, fasting blood sugar, and other biochemical factors have been checked by auto analyzer were recorded from the medical files of studied population. Venous blood sample was obtained from each participant at the start of dialysis session and used for measurement of IL-6 and hs-CRP serum level. The presence of CVD risk factors and level of studied inflammatory factors compared in HD patients with and without CVD and compared in the two groups.

Laboratory measurements
The level of hs-CRP was measured by immunoturbidimetry method (Pars Azmoon kits; Tehran-Iran). The level of IL-6 was measured by enzyme-linked immunosorbent assay method (Orgenium kit; Finland).

Statistical analysis
Data were analyzed using SPSS version 20 (SPSS Inc., Chicago, IL, USA). Studied variables in the two groups were compared using Chi-square, *t*-test, Fisher exact test, and univariate analysis of variance test were used to determine the association between studied risk factors and CVD. Backward stepwise regression analysis and analysis of covariance (ANCOVA) were used to abolish the effect of confounding variables.

RESULTS

In this study, eighty HD patients with ($n = 40$) and without ($n = 40$) history of CVD were enrolled. Demographic characteristics, traditional and nontraditional risk factors, and biochemical factors in two studied groups are presented in Table 1.

Between the two groups, patients with history of CVD were older, had lower body mass index (BMI), and more diabetics than that of without history of CVD ($P < 0.05$). Mean level of high-density lipoprotein cholesterol (HDL-C), calcium, and white blood cell (WBC) were significantly lower in HD patients with CVD ($P < 0.05$). Mean level of IL-6 and hs-CRP were not different significantly between studied groups ($P > 0.05$).

Frequency of patients with IL-6 ≥ 5 pg/mL was 65% ($n = 26$), and 55% ($n = 22$) in patients without and with CVD, respectively ($P > 0.05$). Frequency of patients with

Table 1: Demographic characteristics, traditional risk factors, inflammatory markers, and biochemical factors in hemodialysis patients with and without cardiovascular disease

	HD patients with CVD (*n*=40)	HD patients without CVD (*n*=40)	Total patients (*n*=80)	*P*	OR	95% CI Lower	95% CI upper
Demographics							
Age (years)	61.92±12.20	52.17±15.91	57.04±14.05	0.003	1.051	0.987	1.119
Sex (female/male)	19/21	14/26	33/47	0.18	0.144	0.015	1.403
BMI (kg/m^2)	22.30±2.84	24.47±3.90	23.38±3.37	0.006	0.572	0.371	0.881
Duration of dialysis (months)	30*	25*	27.50	0.59*	0.998	0.972	1.024
Traditional CVD risk factors							
Smoking, *n* (%)	11 (27.5)	10 (25.0)	21 (26.25)	0.56	11.756	1.106	124.988
Diabetes, *n* (%)	28 (70)	16 (40)	44 (55)	0.006	16.750	1.428	196.480
Hypertension, *n* (%)	16 (40)	12 (30)	28 (35)	0.24	0.400	0.048	3.355
Hyperlipidemia, *n* (%)	6 (15)	4 (10)	10 (12.5)	0.36	6.758	0.174	262.708
Systolic blood pressure (mmHg)	131.02±19.33	128.82±19.29	129.92±19.31	0.91	1.045	0.978	1.117
Diastolic blood pressure (mmHg)	76.40±12.75	75.85±12.85	76.12±12.80	0.84	0.982	0.889	1.084
Inflammatory factors							
IL-6 (pg/ml)	10.5*	28.5*	19.50	0.403*	1.001	0.997	1.006
hs-CRP (mg/l)	7.42±4.45	14.27±6.79	10.84±5.62	0.15	0.998	0.954	1.0044
Biochemical measurements							
FBS (mg/dl)	129.27±60.56	111.92±45.85	120.59±53.2	0.15	1.003	0.985	1.021
Hb (g/dl)	10.27±1.47	9.79±1.95	10.03±1.71	0.22	1.431	0.773	2.652
WBC (n/mm^3)	5886.0±2012	7076.50±2644.8	6481.25±2328.4	0.02	1.000	0.999	1.000
Cholesterol (mg/dl)	147.78±37.30	161.95±39.81	154.86±38.55	0.11	0.984	0.937	1.034
Triglyceride (mg/dl)	136.64±63.78	154.02±56.51	145.33±60.14	0.20	0.977	0.952	1.002
HDL (mg/dl)	38.58±7.12	43.42±7.43	41±7.27	0.005	0.826	0.685	0.998
LDL (mg/dl)	90.36±27.46	90.76±24.53	90.56±25.99	0.89	1.069	0.983	1.163
Alb (g/dl)	3.60±0.51	3.71±0.50	3.65±0.50	0.44	3.152	0.212	46.781
PTH (pg/ml)	431.88±326.62	437.43±346.79	434.65±336.7	0.95	1.001	0.999	1.002
Ca (mg/dl)	8.37±0.99	8.90±0.76	8.63±0.87	0.009	1.178	0.039	0.816
P (mg/dl)	4.34±0.96	4.34±1.11	4.34±1.03	0.98	0.824	0.336	2.023
Fe (μg/dl)	76.93±62.20	63.54±47.23	70.23±54.71	0.34	0.996	0.990	1.002
TIBC (μg/dl)	306.05±181.80	316.58±213.79	311.31±197.79	0.81	1.002	0.997	1.007
Ferritin (ng/ml)	186*	204*	195	0.91	1.004	0.998	1.003

*Median. IL-6 = Interlukin-6; hs-CRP = High-sensitivity C-reactive protein; FBS = Fasting blood sugar; WBC = White blood cell; HDL = High-density lipoprotein; LDL = Low-density lipoprotein; Alb = Albumin; PTH = Parathyroid hormone; Ca = Calcium; P = Phosphorous; Fe = Iron; TIBC = Total iron binding capacity; BMI = Body mass index; CVD = Cardiovascular disease; Hb = Hemoglobin; HD = Hemodialysis; OR = Odds ratio; CI = Confidence interval

hs-CRP ≥ 10 mg/L was 30% (*n* = 12) in the two studied groups (12 patients in each group) (*P* > 0.05).

In backward stepwise regression analysis from studied variables including age, diabetes mellitus (DM), BMI, WBC, HDL-C, IL-6 and hs-CRP, only age (odds ratio [OR] =0.985, 95% confidence interval [CI], 0.921–0.996, *P* = 0.03), DM (OR = 0.198, 95% CI, 0.057–0.689, *P* = 0.011), BMI (OR = 1.345, 95% CI, 1.102–1.641, *P* = 0.04), and HDL (OR = 1.091, 95% CI, 1.003–1.687, *P* = 0.042) were significantly different between the two groups.

Univariate analysis of variance showed that there was not any significant relationship between IL-6 and hs-CRP with traditional CVD risk factors such as BMI, HDL-C, and cholesterol. There was significant positive correlation between hs-CRP and IL-6 (*P* < 0.001, *r* = 0.408).

DISCUSSION

In this study, the presence of traditional CVD risk factors and level of inflammatory markers were compared in two groups of chronic HD patients with and without CVD. The findings indicated that there was not any significant difference in the level of IL-6 and hs-CRP in our HD patients with and without CVD.

There are many reports regarding the association between inflammation and CVD in chronic HD patients.[23,24] Both of the studied inflammatory biomarkers, hs-CRP, and IL-6, have been shown to predict CVD in patients with ESRD.[15-18] Although most of the reports confirm their predictive role in this regard, there are also reports which did not indicate the association and there is growing evidence that the relation is not causal.[19,20,25]

In a recent review study on the predictive use of inflammatory factors mainly CRP for CVD, Heidari has concluded that further studies are required to use these factors as predictor factors for CVD and its related mortality in chronic HD.[26]

As mentioned above, these factors have been shown to be a strong predictor of CVD in general population, and the relation between the factors and CVD in ESRD patients have been reported by Zimmermann *et al*.[9] In addition, Ikizler *et al*. confirmed the findings of the previous studies specially for hs-CRP.[27]

In a cohort study conducted by Panichi *et al*. among 218 ESRD patients, the role of IL-6 and CRP as a predictor of CVD and its mortality was evaluated. Only 162 patients could be included in the primary analysis. They showed that cardiovascular mortality relative risk for the increase of 1 standard deviation unit was 2.09 (95% CI 1.52–2.88) for log (IL-6) and 1.66 (1.23–2.24) for log (CRP). With adjustment with the other factor, the RR became 1.90 (0.18–2.82) for log (IL-6) and 1.16 (0.81–1.66) for log (CRP). The results show that IL-6 is better predictor than CRP for this purpose.[28]

There are some possibilities which could explain the superiority of IL-6 to CRP in this field. IL-6 is located on the upstream of the cascade of many acute-phase reactants synthesis including CRP.[29] Moreover, evidence demonstrated that toxic effects of IL-6 for developing or progressing CVD are stronger than CRP.[30]

Zhang *et al*. have investigated the relation between CRP haplotype with its serum level as well as incident CVD risk among dialysis patients. They showed that the haplotype could predict the level of CRP but not CVD risk in that population.[31]

Kaysen *et al*. have reported that CRP or IL-6 are not substituted for all inflammatory cardiovascular risk factors in HD patients.[32]

The findings of a review study by Stenvinkel *et al*. emphasized the role of gene polymorphism in renal epidemiology and role of these factors as a predictor of CVD in uremic patients.[7]

In this study, the level of both hs-CRP and IL-6 was lower in patients with CVD. However, this difference proved to be statistically insignificant. HD patients with CVD were older and had lower BMI than those without CVD. Obviously CVD develops during time so it is not surprising that CVD patients be older, but lower BMI in this group maybe is a sign of malnutrition. As reported previously, the level of inflammatory marker in HD patients may vary over time due to patients'-related factors including genetic determinants, residual renal function, and dialysis-related factors.[33,34]

In accordance with known deleterious effect of diabetes, we showed that diabetes was more frequent in HD patients with CVD than those without CVD. Although we did not determine that whether all of the HD patients were cases with diabetic nephropathy, according to the findings of Irbesartan Diabetic Nephropathy Trial, CRP could not be a predictor for CVD in type 2 diabetic patients with diabetic nephropathy.[35]

In our study, level of HDL-C was higher in HD patients without CVD, and it seems that this factor may provide protection from CVD. HD patients with CVD had lower WBC count. This may show lower inflammatory status in this group as similarly indicated by lower IL-6 and hs-CRP serum levels. Although the details of drug history were not obtained from the studied population, it seems that most of the patients were under treatment of hyperlipidemia or CVD. There are reports about the anti-inflammatory effect of statins, the most commonly used agent for dyslipidemia, on CRP level. Many studies have shown the reduction of inflammatory markers during the treatment of dyslipidemic HD patients with statins.[36,37]

After controlling the effect of confounding factors including age, BMI, DM, serum calcium, and HDL-C on hs-CRP, and IL-6 using ANCOVA test, there was not any significant association between the two groups.

The main limitation of this study was the small sample size of studied population. It seems that a prospective study with patients who newly undergo HD would be more helpful for determining the predictive value of hs-CRP and IL-6 factors on cardiovascular mortality and morbidity.

CONCLUSION

The findings indicated that the level of inflammatory factors including hs-CRP and IL-6 are not significantly different in HD patients with and without CVD. However, for obtaining more definite conclusion in this field and evaluation their predicting role in this field, it is recommended to study other novel inflammatory markers as well as the additive effect of the inflammatory factors with traditional ones in larger sample size and longer follow-up.

Conflicts of interest
There are no conflicts of interest.

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