The Levels of Inflammatory Markers (IL-6, CRP), Homocysteine and Lipid Profile in Hemodialysis Patients, Isfahan

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Abstract

**Background:** Cardiovascular disease (CVD) and inflammation are among the most common causes of mortality in dialysis patients. Factors such as chronic inflammation, dyslipidemia and elevated homocysteine levels may underlie the increased risk of CVD.

**Objectives:** This study aimed to investigate levels of inflammatory markers, homocysteine, lipid profile, WBC, and BMI levels in hemodialysis patients compared with the control subjects in Isfahan.

**Methods:** A total of 32 hemodialysis patients and 32 healthy subjects were selected randomly and their serum levels of IL-6, CRP, homocysteine, and lipid profiles were measured.

**Results:** According to the results, most of the studied hemodialysis patients compared to the healthy subjects had non-normal amounts of IL-6, CRP, and homocysteine i.e., higher than healthy subjects (P < 0.05). Cholesterol, HDL, and LDL were significantly lower than the healthy subjects (P < 0.05), while triglycerides and VLDL levels showed no significant difference (P: 0.38 > 0.05). A significant difference of BMI was observed between the normal subjects and the patients; thus, this factor in patients was less than what was in the healthy group (P: 0.001 < 0.05).

**Conclusions:** The results demonstrated that in most patients the levels of factors IL-6, CRP, and homocysteine were abnormal compared to the healthy group. In addition, the risk of developing cardiovascular and inflammatory disease as well as dyslipidemia in these patients is higher than normal.

Keywords: Hemodialysis, IL-6, CRP, Homocysteine, Lipid Profile

1. Background

Chronic kidney disease (CKD) means lasting and irreversible kidney damage that can get worse over time and eventually lead to end-stage renal disease (ESRD), which requires one of the renal replacement therapies including dialysis or transplantation (1). CVD are one of the most frequent causes of death in dialysis patients. Factors such as blood lipid disorders, chronic inflammation, hypertension, oxidative stress, elevated homocysteine level, anemia, and mineral metabolism disorders may be attributed to elevated CVD rate.

Another factor that could contribute to mortality among hemodialysis patients is systemic inflammation (2). IL-6 is a proinflammatory pleiotropic cytokine that involves many body biological activities and regulation of immune responses and inflammatory reactions by inducing the hepatic synthesis of acute phase proteins such as CRP, serum amyloid, fibrinogen, hepcidin, haptoglobin, and antikimotropsin, as well as by decreasing production of albumin, cytochrome p450, fibronectin, and transferrin (3).

CRP as a sensitive but non-specific index of the acute phase, serves as the most commonly used marker of chronic inflammation. CRP synthesis and secretion are done in response to pro-inflammatory cytokines, particularly IL-6 (4).

Dyslipidemia is another factor that may be associated with increased risk of CVD in dialysis patients. Generally, dyslipidemia is said to be a disorder in the lipid composition of the blood, including the levels of triglyceride, cholesterol, LDL-C, and HDL-C (5).
3. Methods

The present case-control survey of 32 hemodialysis patients and 32 healthy controls (men and women in variable age distribution) were undertaken to evaluate the serum levels of inflammatory factors (IL-6, CRP), homocysteine, lipid profile cholesterol, triglyceride, HDL, LDL, VLDL as well as WBC and BMI levels in the Amiralmomenin Center in Isfahan, 1396. The number of samples were selected based on the sample size formula, which is given below. We excluded participants with a history of heart diseases, acute illnesses, history of immunologic disease, and steroid therapy from the study. Healthy individuals selected as controls (21 males and 11 females) had no history of disease and no specific drug consumption. It was attempted that age, sex, and race distribution of these two groups were similar. Weights and heights were measured to calculate body mass index (BMI) by dividing weight in kilograms by the square of height in meters.

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N = \frac{(Z_1 + Z_2)^2 \cdot 2 \cdot S^2}{d^2}
\]

\[Z_1 = 1.96, Z_2 = 1.84, d = 0.7 \cdot s\]

3.1. Laboratory Measurements

Subjects provided written informed consent. In the morning of the monthly visit, venous blood samples of hemodialysis patients were taken after 12-h of fasting. After separating the serum, the level of IL-6 was measured by the enzyme linked immunosorbent assay method (Oragenium kit; Finland). The level of homocysteine in samples was measured by the Axis-Shield Homocysteine EIA kit (UK) with the ELISA method. Pars-Azmun kits along with BT2000 auto-analyzer were used to obtain lipid profile levels. Serum CRP concentrations were determined via MindrayBS800 auto-analyzer and Pars-Azmun kits using the immunoturbidimetric method.

3.2. Statistical Analysis

Data analysis was performed with SPSS version 19 and the level of significance was set at P < 0.05. In order to compare the average of each factor in healthy and patient groups by the Kolmogorov-Smirnov test, the data distribution normalcy of each factor in both groups was evaluated. In the case of normality, independent-samples t-test, otherwise the Mann-Whitney U-test was used. Factors IL-6, CRP, Hcy, cholesterol, LDL, and WBC were not normally distributed, however, BMI, triglycerides, HDL, and VLDL were normal.

4. Results

In this study, hemodialysis patients without a history of CVD (n = 32) and the healthy group (n = 32) were enrolled. Demographic characteristics in two studied groups are presented in Table 1.

Table 1. Demographic Characteristics in Hemodialysis Patients and the Healthy Group

| Demographics            | Hemodialysis Patients (N = 32) | Healthy Group (N = 32) |
|-------------------------|--------------------------------|------------------------|
| Age, y                  | 18 - 78                        | 18 - 78                |
| Sex (female/male)       | 11/21                          | 11/21                  |
| Systolic blood pressure, mmHg | 128.02 ± 19.30               | 120.32 ± 19.09         |
| Diastolic blood pressure, mmHg | 75.60 ± 12.85                | 75.45 ± 12.05          |

There were no significant differences between both patient and control groups in age and sex. According to the obtained results, most of the studied hemodialysis patients compared to the healthy individuals, had abnormal amounts of IL-6, CRP, and homocysteine. Serum levels of IL-6, CRP, and homocysteine were higher than those in the normal group (P value < 0.05). However, cholesterol lipid profile, HDL, and LDL were significantly lower in the patient group than the control group (P value < 0.05). Serum levels of triglycerides and VLDL in patients compared to healthy controls were higher, however, no significant difference was seen (P value 0.38 > 0.05). In the patients, BMI was significantly less than healthy individuals (P: 0.001 < 0.05). WBC did not show any statistically significant difference between two groups (P: 0.84 > 0.05). Table 2 shows the results of the studied factors.

Table 2. The Results of Biochemical Tests in Patients and Control

|  | Patients (N = 32) | Control (N = 32) | P Value |
|  |                 |                |         |
| IL-6, pg/ml               | 23.77 ± 44.93 | 7.41 ± 15.53 | 0.00    |
| Hcy, µmol/l               | 15.69 ± 5.05 | 9.29 ± 5.78  | 0.00    |
| CRP, mg/l                 | 25.47 ± 10.13 | 10.23 ± 8.37 | 0.00    |
| Chol, mg/dl               | 142.91 ± 32.35 | 191.09 ± 41.13 | 0.00  |
| TG, mg/dl                 | 156.28 ± 84.93 | 39.59 ± 67.06 | 0.38    |
| HDL, mg/dl                | 34.47 ± 6.83  | 47.5 ± 8.54  | 0.00    |
| LDL, mg/dl                | 76.68 ± 32.41 | 115.67 ± 37.36 | 0.00  |
| VLDL, mg/dl               | 31.26 ± 18.99 | 27.92 ± 13.41 | 0.38    |
| WBC, 10³/µl               | 6965.61 ± 2721.08 | 6609.38 ± 1762.88 | 0.84  |
| BMI, kg/m²                | 22.72 ± 6.43  | 21.38 ± 3.88  | 0.001   |

Abbreviation: Hcy, homocysteine.
5. Discussion

In our study, IL-6 and CRP serum, which are important indications of inflammation in the body, were evaluated in both hemodialysis patients and healthy controls. The results showed high levels of IL-6 and CRP in patients compared to the healthy individuals. According to the researches such as Babaie et al. (6), and Rysz et al. (7), these results were not unexpected. The inflammation caused by increasing concentrations of mentioned factors could bring about many complications including cardiovascular diseases, which are the main cause of mortality among patients with renal insufficiency like hemodialysis patients (7).

The inflammation causes in patients who are undergoing dialysis are not well known. Prior studies have indicated that in hemodialysis patients the levels of proinflammatory cytokines have been 8 to 10 times higher than in healthy control subjects (8). The available data suggest that among these factors IL-6 plays a key role in promoting inflammatory events in uremic patients through the activation and proliferation of lymphocytes, B-cell differentiation, leukocyte recruitment, and the induction of an acute phase protein response in the liver (9). In these patients, inflammation and excessive oxidative stress could be due to reduced excretion of cytokines and other inflammatory compounds, contact of white blood cells with membrane of dialysis filtration, especially bioincompatible membrane, pollution of solutions used for hemodialysis, infection of locations of hemodialysis vascular access, the loss of water-soluble antioxidants, and prooxidants accumulation in the body (10, 11).

The CRP is an inflammatory marker where its level in patients with acute inflammatory events increases (12). A number of studies such as Pecoits-Filho et al.’s surveys (13) have demonstrated a significant relationship between IL-6 and CRP which, as previously mentioned, IL-6 is in the beginning of the cascade of events leading to the synthesis of acute phase reaction. Therefore, a direct relationship between IL-6 and CRP is predictable and proven in numerous studies. Thus, to improve the outcomes in hemodialysis patients, physicians, especially nephrologists, should pay special attention to the discovery of subclinical inflammation causes and its potential treatments.

In this study, the obtained results suggested a significant increase of homocysteine in patients compared to healthy subjects. So far, studies have shown that increased plasma Hcy is one of the major complications of hemodialysis and that its amount elevated coinciding with the decrease of active renal mass, which leads to the decreased kidney glomerular filtration rate (GFR), while its level declines at the start of dialysis but does not place in the normal range. Thus, hyperhomocysteinemia is frequently observed in at least 85% - 100% of dialysis patients and regarded an independent cardiovascular risk factor in these patients (14). In addition to the above study, the research results of Ducloux et al. (15), illustrated that in hemodialysis patients, serum homocysteine levels were higher compared to the normal subjects; these results are in agreement with our findings. As shown in Table 3, homocysteine is only correlated with LDL.

Hemodialysis patients usually have high TG, decreased HDL levels, and an increase of lipoprotein a. Total cholesterol and LDL in these patients usually remains within the normal range. Cholesterol levels in them may decline and there will be an inverse relationship between mortality and cholesterol concentration (16).

The results of this study demonstrate that qualitative changes in the lipid compositions or endothelium are more important than their overall plasma levels. In addition, the results show an increase in serum triglycerides levels in patients undergoing hemodialysis compared to healthy subjects. Different studies such as (17, 18) confirm the results obtained in this study. The main causes expressed for the increase of triglyceride in chronic dialysis patients are plasma clearance of triglycerides and increased hepatic triglyceride synthesis. Elevated plasma triglyceride levels mainly contribute to its reduced clearance as a result of structural changes of triglycerides and reduced activities of lipoprotein lipase and hepatic lipase. Factors such as increased levels of parathyroid hormone and existence of lipase inhibitor in the blood circulation are introduced for the increase of triglyceride (19).

HDL, another risk factor examined in our survey, prevents cholesterol accumulation in tissue and acts as an antioxidant. CAD is the leading cause of death worldwide. Increase of LDL and its influence in the endothelial cells along with their oxidation caused by free radicals and structural changes give rise to the processes, leading ultimately to the atherosclerosis (20).

Our obtained results revealed decreased serum levels of HDL in hemodialysis patients compared to the healthy controls, which is a sign of dyslipidemia and prevalence of atherosclerosis in these patients. Numerous studies support this finding and present a variety of reasons for reducing the amount of HDL in hemodialysis patients, such as low physical activity, high blood pressure, and malnutrition (19). One reason of decreased HDL in our patient group was reduced carnitine synthesized by the kidneys. Carnitine is an essential vitamin-like nutrient for humans that play an important role in fatty acids metabolism. In another study with L-carnitine prescription, the serum levels of HDL increased (17).

In this research, decreased serum levels of LDL in
Table 3. The Relationship and Correlation Between Examined Factors

| Control/Group (N = 32) | Pearson Correlations |
|------------------------|----------------------|
|                        | IL-6                 | CRP       | Hcy       | TG        | CHOL      | HDL       | LDL       | VLDL      | WBC       |
| IL-6                   |                      |           |           |           |           |           |           |           |           |
| Correlation            | 1                    | -0.159    | 0.285     | -0.388    | -0.337    | -0.227    | 0.285     | 0.557     |
| Sig.                   | 0.000                | 0.386     | 0.144     | 0.103     | 0.059     | 0.211     | 0.114     | 0.001     |
| CRP                    | 0.409<sup>b</sup>    | 1         | -0.063    | 0.440<sup>b</sup> | -0.256 | -0.400<sup>b</sup> | -0.367<sup>b</sup> | 0.440<sup>b</sup> | 0.569<sup>a</sup> |
| Sig.                   | 0.020                | 0.734     | 0.012     | 0.157     | 0.023     | 0.039     | 0.012     | 0.001     |
| Hcy                    | -0.293               | -0.144    | 1         | -0.078    | -0.293    | -0.148    | -0.220    | -0.078    | -0.253    |
| Sig.                   | 0.104                | 0.431     | 0.670     | 0.104     | 0.418     | 0.227     | 0.669     | 0.163     |
| TG                     | -0.300               | -0.274    | -0.011    | 1         | -0.036    | -0.448<sup>b</sup> | -0.446<sup>b</sup> | 10.000<sup>a</sup> | 0.370<sup>b</sup> |
| Sig.                   | 0.096                | 0.129     | 0.954     | 0.844     | 0.011     | 0.011     | 0.000     | 0.037     |
| CHOL                   | -0.295               | -0.269    | 0.299     | 0.233     | 1         | 0.634<sup>a</sup> | 0.894<sup>b</sup> | -0.036    | 0.066     |
| Sig.                   | 0.101                | 0.136     | 0.096     | 0.199     | 0.000     | 0.000     | 0.844     | 0.722     |
| HDL                    | 0.073                | 0.034     | -0.092    | -0.062    | 0.395<sup>b</sup> | 1         | 0.675<sup>a</sup> | -0.448<sup>b</sup> | -0.130    |
| Sig.                   | 0.691                | 0.852     | 0.617     | 0.737     | 0.025     | 0.000     | 0.010     | 0.480     |
| LDL                    | -0.234               | -0.206    | 0.354<sup>b</sup> | -0.088    | 0.927<sup>a</sup> | 0.229    | 1         | -0.446<sup>b</sup> | -0.086    |
| Sig.                   | 0.197                | 0.258     | 0.047     | 0.632     | 0.000     | 0.208     | 0.011     | 0.618     |
| VLDL                   | -0.300               | -0.274    | -0.011    | 10.000<sup>a</sup> | 0.233 | -0.062    | -0.088    | 1         | 0.370<sup>b</sup> |
| Sig.                   | 0.096                | 0.129     | 0.954     | 0.000     | 0.199     | 0.737     | 0.632     | 0.037     |
| WBC                    | 0.174                | 0.152     | -0.085    | -0.208    | -0.412<sup>b</sup> | -0.024    | -0.374<sup>b</sup> | -0.208    | 1         |
| Sig.                   | 0.341                | 0.406     | 0.645     | 0.254     | 0.019     | 0.895     | 0.035     | 0.254     |

Abbreviations: CHO, cholesterol; Hcy, homocysteine.
<sup>a</sup>Correlation is significant at the 0.01 level (2-tailed).
<sup>b</sup>Correlation is significant at the 0.05 level (2-tailed).

Hemodialysis patients compared to the healthy subjects were recognized. In some of the studies (17, 21) no significant differences in LDL serum levels were found between hemodialysis patients and healthy individuals. Other studies obtained results similar to the results of our study concerning the LDL levels (22, 23). This research demonstrated that kidney failure did not affect the lipid profile and would have the greatest impact on apolipoproteins. Also, malnutrition and prevalence of dyslipidemia were announced by them as the primary causes of reducing LDL in hemodialysis patients. However, other studies faced with increased LDL levels in dialysis patients (24). The difference in LDL levels may be related to either the number of patients or primary cause of kidney failure in the patients. If the cause of kidney failure would be diabetes, lupus, or glomerulonephritis, the primary disease would also affect lipid profile. Regarding to this fact that most blood cholesterol (70%) is transferred by LDL and the significant relationship between serum cholesterol and serum LDL levels in dialysis patients is one of reasons LDL reduction can be linked to the cholesterol decline.

The results of this study showed increased serum lev-
levels of VLDL in patients undergoing hemodialysis compared to healthy controls, however, this increase was not significant. In other studies, no significant differences in relation to serum levels of VLDL between hemodialysis patients and healthy people have been seen (17, 21). Nevertheless, most studies have reported that in patients with chronic renal failure undergoing hemodialysis the amounts of VLDL increased (25).

Our findings implied reduction of cholesterol levels in hemodialysis patients compared to healthy individuals. Although many studies have shown increased cholesterol levels, cholesterol levels may decrease in hemodialysis patients and there will be an inverse relationship between mortality and cholesterol concentration in such patients (17). The vast majority of cholesterol in the human body is supplied through food intake; therefore, given the fact that most CKD patients have malnutrition, cholesterol lowering in these patients than normal healthy people appears natural. In addition to the malnutrition, probable liver disease and renal clearance can be underlying risk factors of lowered cholesterol in these patients (26).

Worsening kidney function is involved in increasing infection and various immune disorders. In addition, frequent dialysis in these patients leads to activation of leukocytes and subsequent production of the cytokine. Increase of WBC in them results in graph and fistula infections as well as increased inflammatory responses. In relation to the number of WBC in hemodialysis patients, few studies have been conducted; however, the findings of this study are in agreement with the results of Tetta et al. (11). Increase of CRP levels was correlated with the WBC and IL-6 levels in patients. In both patients and healthy groups a notable correlation between cholesterol and LDL and also between TG and VLDL levels were observed. Patients in the present survey had a BMI lower than the control group. This result that was consistent with another study demonstrated the increased prevalence of malnutrition in these patients (9). BMI was not significantly associated with any of other factors.

5.1. Conclusions

Chronic renal failure (CRF) or CKD occurs due to progressive irreversible nephron erosion whose end-stage treatments are either dialysis or kidney transplant. CVD is the major cause of morbidity and mortality in CRF patients, including hemodialysis patients as it accounts for almost 50% of deaths in the ESRD population. Moreover, the prevalence of CVD is three to four times higher in these patients than in the general population (27). Known risk factors of CVD such as lipid abnormalities, hypertension, and diabetes are common in hemodialysis patients; however, they cannot justify the high prevalence of CVD in these patients (11).

The current study was carried out to assess factors IL-6, CRP, homocysteine, lipid profiles, WBC, and BMI that likely have an effect on the course of disease in patients with CRF including hemodialysis patients. Based on the results obtained, it appears that hemodialysis patients in relation to inflammatory factors (CRP, IL-6) are in a relatively bad condition and in relation to lipid profile they ahead with dyslipidemia. Our findings imply that these patients are at risk of CVD and therefore, improvement of nutritional status as well as water quality and other dialysis-related factors seem necessary. One of the major limitations of this study is the elimination of dialysis patients with a history of heart disease, acute illnesses, history of immunologic disease, and patient with specific medications, which reduced the number of available samples for this research. Therefore, it is recommended that increasing the number of samples can provide more accurate and better results.

Supplementary Material

Supplementary material(s) is available here [To read supplementary materials, please refer to the journal website and open PDF/HTML].

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Footnotes

Authors’ Contribution: Elnaz Sabzevari, Kahin Shahnipour and Aliasghar Moshtaghie: study concept and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, statistical analysis, administrative, technical, and material support.

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