Effect of Hepatitis C Virus on C-Reactive Protein and Interleukin-6 in Hemodialysis Patients

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Introduction. Patients with end-stage renal disease are at a high risk of hepatitis C virus (HCV) infections. These patients are on maintenance hemodialysis where they are exposed to dialysis fluid and dialysis membrane that generate an acute-phase response, which leads to inflammation, reflected in increased inflammatory markers like C-reactive protein (CRP) and interleukin-6 (IL-6). The aim of this study was to investigate levels of IL-6 and CRP in patients on hemodialysis and to determine effects of HCV on these markers.

Materials and Methods. A total of 43 patients (39.5% men and 60.5% women; age range, 21 to 65 years) on maintenance hemodialysis for a period of at least 3 months were included. Twenty-four of them were HCV positive. Serum IL-6 and CRP were assessed in all patients.

Results. Of HCV-positive patients, 11 (45.8%) had high levels of CRP, while 13 (54.2%) had low levels and increased levels of IL-6 (1064 ± 544.2 pg/mL, P = .19). Of 19 HCV-negative patients, 13 (68.4%) had high levels of CRP, while 6 (31.6%) had low levels, and all these patients had low levels of IL-6, as compared to HCV-positive patients. No significant correlation was observed between the levels of CRP or IL-6 and the duration of dialysis.

Conclusions. In our study, we found high serum IL-6 and CRP levels in HCV-positive hemodialysis patients, compared with HCV-negative ones. However, we failed to show the significance of these differences. More studies with large sample sizes and evaluation of the other inflammatory markers are warranted.

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INTRODUCTION

Patients on dialysis are at a high risk for blood-born infections, such as hepatitis B and hepatitis C infection. Infection with hepatitis C virus (HCV) is more common.[1,2] There is a strong relationship between HCV and hemodialysis. Therefore in end-stage renal disease patients, HCV remains a significant cause of morbidity and mortality.[3] Prevalence of HCV infection varies greatly from less than 5% to nearly 60% in different parts of the world. Hepatitis C antibody was documented in 20% to 50% of hemodialysis patients in Brazil, 24.8% in Iran, 12.7% in Turkey, and 18.9% in Saudi Arabia.[4-7] Various studies performed in Asia, where incidence of HCV infection is highest in the world, have greatly contributed to the understanding of the diseases related to the liver.[8,9] In Pakistan, viral hepatitis is a major cause of chronic liver disease and about 4% of the population has become HCV carrier.[10,11] A study in Japan from 16 centers showed

a mortality rate of 18.8% among hemodialysis patients infected with HCV.[12] Deaths in HCV-infected dialysis patients were 41% higher than in HCV-negative dialysis patients.[13]

C-reactive protein (CRP) belongs to pentaraxin family.[14-16] It is produced by the liver in response to several inflammatory mediators, the most important of which is interleukin-6 (IL-6).[17] C-reactive protein is a sensitive but nonspecific inflammatory marker.[18,19] During inflammation, levels of CRP can be increased up to 1000 folds,[20] and as soon as inflammation subsides it comes to normal levels.[21] Recently, CRP has been documented as a predictor of cardiovascular disorders,[4] myocardial infarction, stroke, and sudden heart attack.[22] It was observed that hemodialysis patients who had CRP levels of more than 10 mg/L showed 3.5 times higher mortality rate than patients with normal levels of CRP.[23]

Studies showed that HCV patients who developed good T helper-1 response were able to clear the virus, whereas those who developed T helper-2 response showed chronic HCV infection. Patients with chronic HCV infection showed presence of interferon-γ (INF-γ), tumor necrosis factor-α, IL-8, Granulocyte macrophage colony-stimulating factor, and IL-10, supported by the observation that liver damage on biopsy was proportional to the level of cytokine. Furthermore, patients with chronic HCV with elevated T helper-1 cytokines are less likely to respond to INF therapy. It was concluded that HCV infection lead to activated T-cell responses that INF therapy make partly through diminution of cytokine response.[24]

Interleukin 6 (IL-6) is a cytokine of hematopoetin family,[25, 26] which is synthesized by mononuclear phagocytes, vascular endothelial cells, and fibroblasts in response to IL-1,[27] which is an inflammatory marker, and its level increases in hemodialysis patients.[18,28,29] It is well documented that the immune system of HCV-infected individuals is suppressed, and they have increased tendency of developing diabetes mellitus and tuberculosis. There is diminished antibody response to HCV and it is the reason why earlier diagnostic kits are unable to detect anti-HCV antibodies as compared to currently used third generation immunoassays. Further, in HCV patients, there is macroglobulinemia, which leads to immune complex deposition in various organs. Therefore, inflammatory markers are raised, ie, CRP and IL-6.[30] There is a synergetic effect of CRP and pro-inflammatory cytokines like IL-6 and IL-10 that serves as a strong predictor of cardiovascular disease mortality.[31] All these changes in HCV-infected individuals may directly or indirectly affect cardiovascular disease and especially the heart because there is increased mortality and morbidity in these patients.[30]

The present study was designed to determine the missing link between HCV, inflammatory markers, and kidney disease. Therefore, it was planned to determine the levels of CRP and IL-6 in HCV-positive and HCV-negative patients on hemodialysis. Since the immune system is suppressed during HCV infection,[30] we also tried to evaluate the effect of age in our subjects by dividing them into two age groups.

MATERIALS AND METHODS

This was a cross-sectional study 43 hemodialysis patients aged between 20 and 65 years who were selected from the Department of Nephrology, Sheikh Zayed Hospital, in Lahore. Of the 43 patients, 26 (60.5%) were men and 17 (39.5%) were women. The study was approved by the Advanced Study and Research Board of the University of Health Sciences, in Lahore and the Ethical Committee of Sheikh Zayed Hospital. Written informed consent of each participate was obtained before collection of blood sample.

Both HCV-positive and HCV-negative patients with kidney failure of either gender, between 20 to 65 years of age on dialysis for at the last 3 months were included. Patients with chronic inflammatory disease, autoimmune disorder, acute infection, malignancy, anti-HIV antibody, pregnancy, and hepatitis B infection were excluded from the study. None of the patients were treated with antiviral agents such as interferon and ribavirin. The major reasons of the study population for admission to the hospital and their percentages are summarized in Table 1.

C-reactive protein levels were measured by latex agglutination (ATLAS Medical, London, UK),[32] while IL-6 was determined by an enzyme-linked immunosorbent assay technique (KOMA Biotech, Seoul, South Korea).[33]

Patients were divided into 2 groups based on their age (20 to 44 years and 45 to 65 years). Continuous values were expressed as mean ± standard deviation

Table 1. End-stage Renal Disease (ESRD) Causes in Hemodialysis Patients With and Without a Positive Hepatitis C Virus (HCV) Antibody*

ESRD Cause	HCV		All
	Positive (n = 24)	Negative (n = 19)	
Hypertension	4 (16.7)	2 (10.5)	6 (13.9)
Diabetes mellitus	2 (8.3)	1 (5.3)	3 (7.0)
Glomerulonephritis	1 (4.2)	2 (10.5)	3 (7.0)
Other nephropathies	3 (12.5)	2 (10.5)	5 (11.6)
Mixed causes	10 (41.7)	8 (42.1)	18 (41.9)
Unknown	4 (16.7)	4 (21.1)	8 (18.6)

*Values in parentheses are percents.

and range. Differences between the two groups were analyzed by the Student *t* test. *P* values less than .05 were considered significant.

RESULTS

A total of 24 patients (55.8%) were positive for HCV and 24 (55.8%) were positive for CRP elevation. The mean level of IL-6 was significantly high in hemodialysis patients, and in particular, it was high in HCV-positive patients as compared to HCV-negative patients, but the difference was not statistically significance (Table 2). Among HCV-positives, however, IL-6 levels were significantly higher in younger patents (20 to 44 years) than older patients (Table 3). The mean level of CRP in HCV-negative patients was insignificantly higher, as compared to HCV-positive patients. However, this difference was significant when the two age groups were compared in HCV-positive patients (Table 4).

Table 2. Interleukin-6 Levels in Hemodialysis Patients With and Without a Positive C-Reactive Protein (CRP) in Relation to Hepatitis C Virus (HCV) Positivity

HCV Groups	Interleukin-6, pg/mL		*P*
	CRP Positive (n = 24)	CRP Negative (n = 19)	
Positives	1203 ± 343	1064 ± 544	.32
Negative	1203 ± 543	944 ± 540	.13

Table 3. Interleukin-6 Levels in HCV in Younger and Older Hemodialysis Patients in Relation to Hepatitis C Virus (HCV) Positivity

HCV Groups	Interleukin-6, pg/mL		*P*
	20 to 44 Years (n = 17)	45 to 65 Years (n = 26)	
HCV-positives	1108 ± 576	752 ± 305	.01
HCV-negatives	1175 ± 498	1108 ± 568	.69

Table 4. C-Reactive Protein Levels in HCV in Younger and Older Hemodialysis Patients in Relation to Hepatitis C Virus (HCV) Positivity

HCV Groups	C-Reactive Protein, mg/L		*P*
	20 to 44 Years (n = 17)	45 to 65 Years (n = 26)	
HCV-positives	7.85 ± 14.85	74.18 ± 93.69	.02
HCV-negatives	46.00 ± 36.00	30.13 ± 35.80	.44

No significant correlation was observed between the levels of IL-6 and CRP and the duration of dialysis.

DISCUSSION

In our cohort, HCV-positive patients had lower CRP levels as compared to HCV-negative patients, while IL-6 levels were higher in HCV-positive patients than in HCV-negative patients. The difference in CRP production in HCV positive patients indicated that liver response to IL-6 stimulation might be changed due to HCV infection. Therefore hepatic injury by HCV could be a reason for the disturbance in the production of CRP.[4]

C-reactive protein levels were high in HCV-negative patients compared to HCV-positive patients. Nascimento and colleagues[4] also found that levels of CRP and IL-6 were significantly high in hemodialysis population. In that study, HCV-positive patients had lower levels of high-sensitivity CRP as compared to HCV-negative patients. There was a significant difference in high-sensitivity CRP-IL-6 ratio in HCV-positive patients which might be due to hepatocellular injury that could affect CRP production. We observed 56% of hemodialysis patients had a high level of CRP, while Panichi and coworkers[34] found that 47% of hemodialysis patients had high CRP levels, and in another study, 36% of hemodialysis patients showed high levels of CRP.[35] Moreover, in the present study, it was observed that 46% of the patients had CRP levels greater than 10 mg/L, which is in agreement with the previous study done by Razeghi and colleagues[36] who found a CRP level greater than 10 mg/L in 41% of hemodialysis patients. In the literature, there are multiple reports on CRP levels; in 2008, Adriana and associates[37] also observed 25% of haemodialysis patients had CRP levels greater than 16.7 mg/L.

The levels of both CRP and IL-6 were not significantly different between HCV-positive and

HCV-negative patients, but both of these values were significantly different between the two age groups of HCV-positive patients. Low levels of CRP in HCV-positive patients of both age groups indicated that liver was impaired by HCV, and therefore, CRP value did not give proper extent of inflammation; hence, these patients may gradually develop cardiovascular disorders. Apparent increase in CRP levels in hemodialysis patients indicated inflammation, and therefore, it was designated as a sensitive and independent marker for malnutrition. These findings also matched with the findings of a study done by Nascimento and coworkers.[4]

In the present study, levels of IL-6 in hemodialysis patients were high, which accords with many other studies.[10,34,380-40] In the present study, IL-6 levels were high in HCV-positive patients compared to HCV-negative patients, and similar findings were found in the study of Nascimento and colleagues.[4] Furthermore, serum IL-6 levels were high in CRP-negative patients, but there was no difference in CRP-positive haemodialysis patients. Among the haemodialysis patients, serum IL-6 levels were high in CRP-negative patients as compared to CRP-positive patients, but the difference was not significant. Zumrutdal and coworkers[41] studied the malnutrition inflammatory score, CRP, and IL-6 in HCV-positive and HCV-negative patients on hemodialysis. They concluded that the malnutrition inflammatory score, comorbidities, number of years on dialysis treatment were associated with HCV positivity, but the differences of CRP and IL-6 levels in these two groups were not significant.

In the present study, no correlation between high levels of IL-6 and duration (in months) of renal dialysis was observed, which did not match with the previous studies carried out by Herbelin and coworkers[38] and Kaizu and colleagues.[42] We could not find a correlation between CRP levels and duration of renal dialysis either, whereas Iseki and associates[23] reported that hemodialysis patients with CRP levels of more than 10 mg/L had significantly high mortality rate during 7 years of the study period as compared to those with CRP levels of less than 10 mg/L. Zimmermann and colleagues[32] found that increased CRP levels of more than 7.5 mg/L had a 2.7% higher mortality risk than CRP levels less than 3.3 mg/L.

CONCLUSIONS

Although in this study there were high levels of CRP and IL-6 in HCV-positive hemodialysis patients of both the age groups, we suggest a study on a larger population of hemodialysis patients for the better understanding of the impact of HCV infection on these markers. Furthermore, liver biopsy should be included, because it may assist in estimating the extent of liver injury and its association with HCV status.

CONFLICT OF INTEREST

None declared.

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