Blood Purification

Hemodialysis – Research Article

Blood Purif
DOI: 10.1159/000522120

Received: November 21, 2021
Accepted: January 18, 2022
Published online: February 17, 2022

Fluctuations in Interleukin-6 Levels during Hemodialysis Sessions with Medium Cutoff Membranes: An Analysis on COVID-19 Case Series

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Keywords
Dialysis membrane · Cytokine · Inflammation · Interleukin-6 · COVID-19

Abstract
Introduction: Interleukin-6 (IL-6) is one of the most important mediators of inflammation. It is also the culprit for a severe disease course in COVID-19. While COVID-19 has higher mortality in hemodialysis (HD) patients, medium cutoff (MCO) membranes were previously suggested as promising tools for better patient outcomes by purging inflammatory mediators. The aim of this study was to analyze changes in IL-6 levels of HD patients who were dialyzed via MCO membranes during their COVID-19 treatments. ***Methods:*** This is an observational study on a group of HD patients who were admitted with COVID-19 diagnosis in a university hospital and intermittently dialyzed using MCO membranes during their hospital stay. IL-6 levels of the patients were measured before and after consecutive dialysis sessions by a commercial kit. Measurements were interpreted together with the clinical data. ***Results:*** Nine patients with a total of 54 measurements were evaluated. IL-6 levels were significantly higher in patients who died (median and interquartile ranges [IQRs] of IL-6 levels for patients who died and survived were 112.0 pg/mL [48.3–399.4] and 5.3 pg/mL [2.2–27.4], respectively; $p < 0.001$). In the comparison of changes in IL-6 levels with dialysis sessions, patients who survived had lower post-dialysis levels (median: 4.5 pg/mL; IQR: 2.2–7.6). However, IL-6 levels had a tendency to increase with dialysis sessions in patients who could not survive COVID-19 (median: 237.0 pg/mL; IQR: 53.8–418.2). ***Conclusion:*** This study describes over time variations in IL-6 levels of COVID-19 patients undergoing HD with MCO membranes. The trend for the changes of IL-6 levels during dialysis sessions was not uniform for all patients. Surviving patients had decreasing levels of IL-6 with consecutive dialysis sessions, while nonsurvivors had an increasing trend.

Introduction

Interleukin-6 (IL-6), which is released in response to infections or inflammation, contributes to acute phase responses and immunological reactions. It induces the synthesis of inflammatory markers like C-reactive protein, promotes Th17 differentiation and Th2 response of CD4+

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T cells, and mediates antibody productions [1]. With these properties, IL-6 is the cornerstone of inflammatory response. Clinical picture of COVID-19 may vary from asymptomatic infection to acute respiratory distress syndrome. For the latter, which is generally complicated with the so-called cytokine release syndrome, IL-6 is the main mediator [2]. There have been different experimental strategies to treat inflammatory response in COVID-19, such as steroids, IL-6 receptor blockers, or extracorporeal procedures to remove cytokines [3, 4]. COVID-19 has high mortality among hemodialysis (HD) patients [5], and this may be attributed to immune dysfunction of this group.

HD may result in an increase of pro-inflammatory cytokines [6]. On the other hand, the use of medium cutoff (MCO) membranes as dialyzers was previously shown to reduce inflammatory mediators [7], and there were reports recommending the use of MCO membranes in COVID-19 [8]. The aim of this study was to evaluate the fluctuations of IL-6 levels in a group of COVID-19 patients who were undergoing chronic HD with MCO membranes.

Materials and Methods

Setting and Patients

The study is conducted on a group of maintenance HD patients who were followed up in designated COVID-19 wards of the Cerrahpasa Medical Faculty University Hospital. COVID-19 was diagnosed by polymerase chain reaction from combined oral and nasopharyngeal swabs. Patients who were on HD for more than 3 months and gave informed consent were considered eligible for the study. Critically ill patients who needed intensive care unit support upon hospital admission and those who had concurrent bacteremia were excluded from the study.

Blood Samples and IL-6 Measurement

A total of fifty-four blood samples from consecutive HD sessions of nine maintenance HD patients were analyzed for IL-6 levels. Twenty-seven paired samples were collected from the afferent (venous) line before and after the dialysis sessions. The IL-6 assay for the quantitative determination in human serum was the sandwich electro-chemo-luminescence immunoassay method. For analysis, the Elecsys® IL-6 test kit was used on Cobas-e–602® immunoassay analyzer (Roche Diagnostics, Indianapolis, IN, USA). Analytical sensitivity of the assay was 1.5 pg/mL. Changes in IL-6 levels (Δ) were calculated as pre-dialysis IL-6 level minus post-dialysis IL-6 level.

Clinical Data

Daily measured complete blood count and inflammatory markers were analyzed for all patients. Severity and clinical progress of COVID-19 were also evaluated for all patients. Chronic kidney disease etiologies and comorbidities of the patients were noted.

Dialysis Sessions

Four hours of routine HD sessions were resumed 3 times weekly for all patients in isolated HD units. Patients underwent HD with MCO dialysis membranes (Theranova 400, Baxter™, Deerfield, IL, USA). The dialysate contained following ion concentrations: Na 140 mmol/L, K 2–3 mmol/L, Ca 1.25–1.50 mmol/L, Mg 0.5 mmol/L, and HCO3 32 mmol/L (Renasol BA-310, Fresenius Medical Care, Istanbul, Turkey). Blood flow rates ranged between 300 and 400 mL/min. The dialysate flow rate was constant at 500 mL/min for each patient. Ultrafiltration was programmed according to the patients' volume status. Heparin or low-molecular-weight heparins were used as anticoagulants.

Treatment Modalities

According to the guidelines released by the Turkish Ministry of Health, all patients were treated with favipiravir (2 × 1,600 mg loading dose, followed by 2 × 600 mg for additional 4 days). Nasal oxygen, high-flow oxygen or noninvasive mechanic ventilation support were provided depending on oxygen saturations. Low-dose steroids (e.g., 6 mg dexamethasone) were used as anti-inflammatory drugs in patients with low oxygen saturations. High-dose steroids (e.g., >250 mg methylprednisolone) were added when oxygen levels did not recover with low-dose steroids. Prophylactic anticoagulants (enoxaparin 4,000 IU subcutaneously) were prescribed to avoid thromboemboli.

COVID-19 Severity Score

COVID severity was scored in a scale that involved asymptomatic infection (0 point), upper respiratory tract or gastrointestinal symptoms (1 point), pneumonia (2 points), pneumonia with hypoxemia (3 points), and multisystemic involvement (4 points).

Statistical Analysis

Continuous data were presented with median and interquartile ranges (IQRs), while categorical data were given as percentages. Mann-Whitney U test was used to compare continuous data. Categorical data were compared by Fisher's exact test. Pearson test was used to calculate correlations between parameters. $p < 0.05$ was accepted as the statistical significance cutoff. SPSS version 22 was used for statistical analysis.

Results

Nine patients with a total of 54 measurements were involved in the study. Median age of the patients was 67 years (IQR: 36–77.5). Etiologies for chronic kidney disease include hypertension (3 patients), diabetes (2 patients), glomerulonephritis (2 patients), lupus (1 patient), and unknown etiology (1 patient). All patients could complete their planned dialysis sessions without any complications. Four of the patients who had relatively more severe clinical picture died. The causes of death were sepsis in 2 patients and respiratory failure due to COVID pneumonia in the remaining two.

Table 1. Clinical data and laboratory values of patients (including subgroup analysis)

	All patients (n = 9)	Subgroup analysis		
		survivors (n = 5)	nonsurvivors (n = 4)	p value
Age	67 (36–77.5)	36 (30.5–61.5)	77.5 (69.5–80.2)	0.032
Male sex, n (%)	6 (66.6)	4 (80)	2 (50)	0.37
Comorbidities				
Diabetes	2	0 (0)	2 (50)	0.09
Hypertension	5	1 (20)	4 (100)	0.02
IHD	3	1 (20)	2 (50)	0.37
Rheumatic D	2	1 (20)	1 (25)	0.86
Duration of dialysis, months	17 (4.5–40.5)	25 (4–40.5)	12.5 (5.7–49.2)	0.90
COVID-19 severity	4 (2.5–4)	3 (2–3.5)	4 (4–4)	0.025
Duration of hospital stay, days	26 (14.5–41.0)	26.0 (13.0–31.5)	36 (16.7–51.5)	0.55
High-dose steroids, n (%)	5 (55)	4 (80)	1 (25)	0.11
Hemoglobin, g/dL	8.9 (7.9–9.4)	9.2 (8.4–10.7)	8.3 (6.7–9.1)	0.012
LDH, U/L	405 (282–563)	292 (234.5–592)	425 (349–530)	0.37
CRP, mg/L	50.6 (19.6–98.8)	26.9 (11.0–76.2)	73.9 (33.2–126.8)	0.09
Ferritin, µg/L	540 (389.2–1,651.0)	660.2 (491.4–1,668.2)	485.1 (126.2–1,284.8)	0.15
D-dimer, mg/L	2.2 (1.4–3.1)	2.1 (1.1–5.6)	2.4 (1.5–3.1)	0.88
Procalcitonin, µg/L	0.87 (0.49–2.8)	0.98 (0.35–3.55)	0.84 (0.50–1.91)	0.79
Albumin, g/dL	2.9 (2.5–3.1)	3.0 (2.7–3.1)	2.9 (2.5–3.2)	0.61
Leukocytes, /µL	8,400 (6,300–10,500)	7,850 (4,650–8,525)	10,400 (8,750–12,850)	0.02
Lymphocytes, /µL	650 (400–1,025)	700 (400–950)	500 (400–1,250)	1.0
Neutrophils, /µL	7,200 (4,500–9,175)	6,300 (3,200–7,450)	8,500 (5,750–11,400)	0.06
Urea, mg/dL	170 (119–212)	145.5 (104.2–225.0)	177 (158–212.5	0.35
Kt/V	1.45 (1.38–1.55)	1.52 (1.34–1.59)	1.42 (1.38–1.47)	0.55
Pre-dialysis IL-6, pg/mL	33.8 (5.5–142.4)	6.1 (2.0–25.9)	106.7 (47.6–343.7)	0.001
Post-dialysis IL-6, pg/mL	24.5 (4.0–237)	4.5 (2.2–7.6)	237.0 (53.8–418.2)	0.001

Continuous data are given with median and IQRs. Categorical data are given with percentages. IHD, ischemic heart diseases; D, Diseases; CRP, C-reactive protein.

When all of the 54 measurements, independent of their relations to the dialysis sessions were compared, IL-6 levels were significantly higher in patients who died (median and IQRs were 112.0 pg/mL [48.3–399.4] vs. 5.3 pg/mL [2.2–27.4]; $p < 0.001$). Both pre-dialysis and post-dialysis IL-6 levels were higher in patients who died ($p < 0.001$) (Table 1).

In the comparison of changes in IL-6 levels with dialysis sessions, patients who survived (n = 5) had a positive clearance with dialysis sessions (median ΔIL-6: 2.1 pg/mL; IQR: 1.2–18.2). However, IL-6 levels showed a general tendency to increase with dialysis sessions in patients who could not survive (n = 4) COVID-19 (median ΔIL-6: −39.6 pg/mL; IQR: −133.6–10.8). Changes in pre-dialysis and post-dialysis median IL-6 levels are demonstrated in Figure 1.

Patients who died were older. They had lower hemoglobin levels and higher leukocyte numbers than patients who survived. Albumin levels as well as dialysis adequacy were similar for both groups. Comparison of other laboratory values and clinical data can be found in Table 1. Changes in IL-6 levels with each dialysis sessions for each surviving and deceased patient are demonstrated in Figures 2, 3. Patients who survived had decreasing levels of IL-6 with consecutive dialysis sessions. On the other hand, dialysis sessions potentiated IL-6 secretion in patients who died.

When pre- and post-dialysis IL-6 levels were compared with inflammation-related markers, there were no correlations of IL-6 with C-reactive protein, ferritin, or d-dimer levels. However, pre-dialysis and post-dialysis IL-6 levels were significantly correlated with lactate dehydrogenase (LDH) levels ($r = 0.6$; $p = 0.001$ and $r = 0.49$; $p = 0.009$, respectively). Additionally, both pre-dialysis and post-dialysis IL-6 levels were inversely correlated with hemoglobin levels ($r = -0.47$; $p = 0.013$ and $r = -0.52$; $p = 0.005$, respectively).

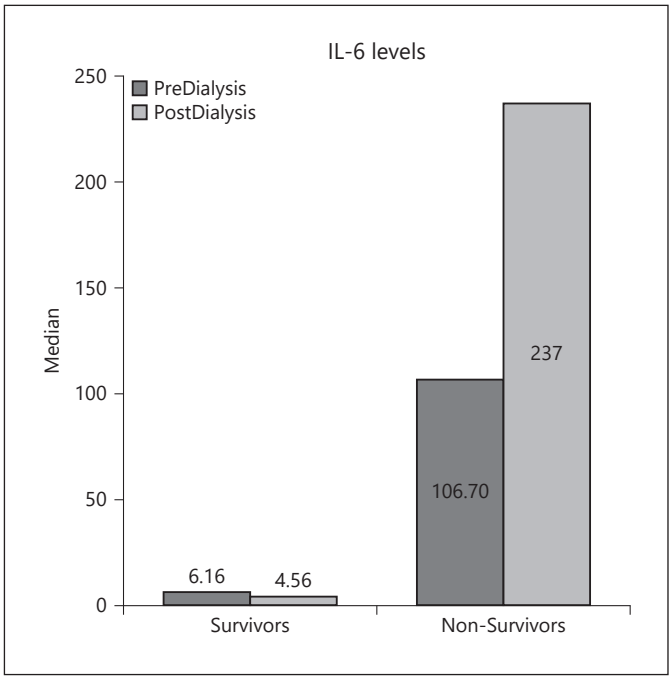

Fig. 1. Comparison of median IL-6 changes in surviving and non-surviving patients.

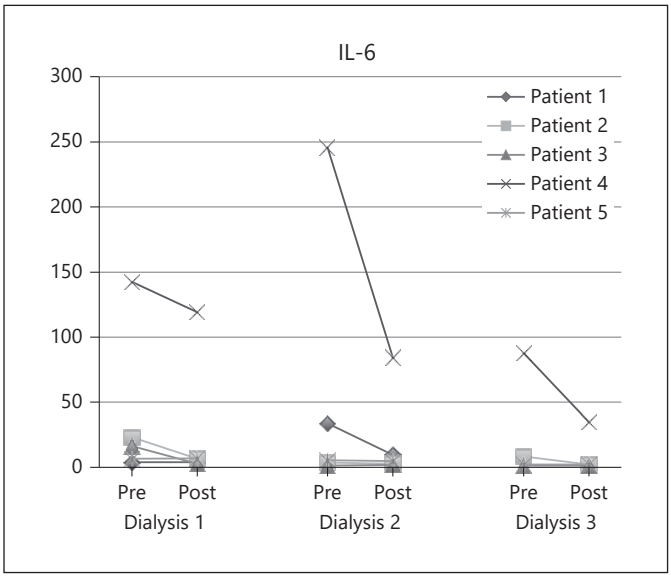

Fig. 2. Changes in IL-6 levels with consecutive dialysis sessions of each surviving patients.

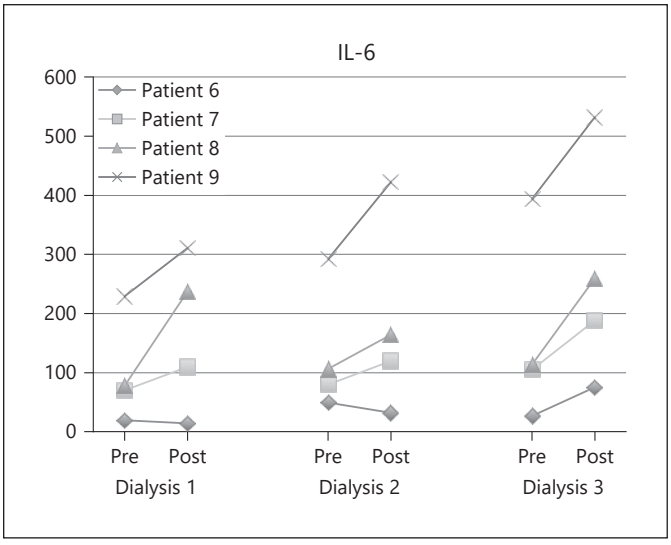

Fig. 3. Changes in IL-6 levels with consecutive dialysis sessions of each patient who died.

Discussion

IL-6 is one of the early mediators of inflammatory response, and it increases earlier and more rapidly than other inflammatory markers [9]. IL-6 also has roles in the course of viral infections [10] and is one of the most important mediators of immunologic response in COVID-19 [11]. Chronic HD patients are already prone to inflammation as pro-inflammatory mediators are released in uremic milieu and their removals are slower [12]. Additionally, each HD session may drive inflammatory response [13]. It was previously shown that IL-6 is associated with long-term mortality in HD patients [14]. As seen in our study, dialysis-related fluctuations in IL-6 levels might also be predictive for short-term mortality in an acute inflammatory condition like COVID-19. Dialysis-related conditions may affect inflammatory response of HD patients and dialysis membranes are among them [15].

MCO membranes were previously suggested to improve survival in COVID-19 patients [16]. This might be possible with cytokine removal benefit of these novel dialyzers. In a study prior to the COVID-19 era, both TNF-alpha and IL-6 mRNA levels decreased with the use of MCO membranes in 4 weeks [7]. We have found that patients who had positive clearance of IL-6 with MCO membranes survived COVID-19. However, this feature was not generalizable to all patients. IL-6 tended to increase with dialysis sessions in some of the patients and these patients had worse prognosis. Those patients who died were older and they also had higher pre-dialysis IL-6 levels. While it was only the hypertension rate that could reach statistical significance, comorbidities were generally more often in these patients as well (Table 1).

Similar to our results, intradialytic increase in IL-6 was found to be associated with mortality in a group of HD patients without any documented infection [17]. However, exact mechanism that increases IL-6 during a dialysis session was not understood. Plausible explanations are effects of dialyzers, bloodstream infections, or water supply-related endotoxins. In our patients, the inflammatory state associated with COVID-19 may be the responsible factor.

There were previous reports underlining the benefit of MCO membranes in HD patients with COVID-19. However, our analysis shows that MCO membranes may have effects in both ways. In some patients, IL-6 is cleared with the dialysis sessions and serial dialysis sessions decrease IL-6 levels. COVID-19 had better prognosis in these patients. In the other group, IL-6 levels had a tendency to increase with dialysis sessions. These patients were older and their COVID-19 courses were relatively more severe. In addition, they had lower hemoglobin levels and higher white blood cell counts. While inflammatory responses are generally expected to decrease in older patients, relatively more severe COVID-19 in these patients might have boosted the inflammatory response. A similar report also found increasing IL-6 levels in HD patients who could not survive COVID-19 [18]. While patients who died were in similar age with the survivors in that study, their COVID-19 was also more severe. Higher white blood cell and neutrophil counts might be consequences of higher IL-6 levels and they also indicate more severe disease course [19]. Although it did not show statistical significance, the use of high-dose steroids was more often in the surviving patients. In a previous study on COVID-19 patients, decreasing IL-6 levels with the use of steroids was suggested to improve prognosis [20]. Dialysis adequacies of our patients who died and survived were similar. In addition, we have not observed any pre- and post-dialysis clinical differences that can be attributed to dialysis sessions.

IL-6 levels were not related to other inflammatory markers. But, there was a positive correlation with LDH levels. LDH is most probably increased as a result of cytokine mediated organ injuries and this may define the correlation with IL-6 levels [21]. On the other site, hemoglobin levels were negatively correlated with IL-6 levels. This might be related to better tissue oxygenation with higher hemoglobin levels and immune-modulatory effects of hemoglobin [22]. Thus, ensuring proper anemia management in HD patients might help for better COVID-19 prognosis. We have not measured dialysis-related changes in other inflammatory markers, as IL-6 is the major mediator of inflammatory response in COVID-19 and is also responsible for the release of other markers.

This study has some limitations, small sample size being the first. Additionally, we have not used other types of dialyzers for a possible comparison with MCO membranes. It should also be underlined that this study was not planned as a formal clearance study and we have not carried out analysis on neither dialysate nor simultaneous pre- and post-filter samples. Future comparative studies with wider samples might be designed to reinforce the findings here.

In conclusion, IL-6 levels are higher in HD patients who could not survive COVID-19. While MCO membranes are promising in the clearance of IL-6, different patient and disease characteristics may affect this property.

Statement of Ethics

Written informed consent was obtained from patients to participate in the study. The study was approved by the Institutional Review Board for Medical Research at Cerrahpasa Medical Faculty (Nr. April 21, 2020/A-10) and by the National COVID-19 Research Supervision Committee (Nr. 2020-05-08T17_04_43). The study was conducted in accordance with the principles of Declaration of Helsinki.

Conflict of Interest Statement

C.R. has been consulting or part of advisory boards for ASAHI, Astute, Baxter, Biomerieux, B. Braun, Cytosor-bents, ESTOR, FMC, GE, Jafron, Medtronic, and Toray. Other authors have no conflicts of interest to declare.

Funding Sources

The Scientific Research Projects Coordination Unit of Istanbul University-Cerrahpasa funded this project (Project Number: THZ-2021-35,756).

Author Contributions

A.M. and M.R.A. did the literature search and designed the study. D.K. performed all laboratory tests. A.M. and S.F.Y. collected the patient data and did the statistical analysis. A.M. and M.R.A. were responsible for funding and project management. C.R. provided technical guidance, critically revised the literature, and interpreted the data. A.M. drafted the manuscript. All authors commented on consecutive versions of the draft and approved the final version.

Data Availability Statement

The dataset is available from the corresponding author on a reasonable request.

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