

Brief Report

Blood Serum Levels of IL-2, IL-6, IL-8, TNF- α and IL-1 β in Patients on Maintenance Hemodialysis

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Cytokines are essential mediators of immune response and inflammatory reactions. Patients with chronic renal failure (CRF) commonly present with abnormalities of immune function related with impaired kidney function and the accumulation of uremic toxins in addition to bioincompatibility of dialyzer membranes. During a hemodialysis (HD) session, cytokines are released mainly by monocytes activated by endotoxin-type compounds in dialyzer fluid, complement factors and direct contact with dialyzer membrane. The study included 15 CRF patients, aged 36.4 ± 2.9 years, on regular HD maintenance therapy for mean 68 ± 10 months and 15 healthy controls. It was designed to assess serum levels of a panel of inflammatory cytokines: IL-1 β , IL-2, IL-6, IL-8 and TNF- α in CRF patients on regular maintenance HD before, 20, 60 and 240 minutes of a single HD session in parallel with C-reactive protein (CRP) as an additional parameter. CRP concentration was increased in HD patients when compared with healthy controls. The concentrations of IL-1, IL-6, IL-8 and TNF- α were increased, whereas the serum level of IL-2 was not altered during a single HD session. *Cellular & Molecular Immunology*. 2006;3(2):151-154.

Key Words: cytokine, chronic renal failure, hemodialysis

Introduction

Cytokines as polypeptide or glycopeptide molecules are the essential mediators of immune response and the inflammatory reactions in addition to numerous biological reaction they are involved in. They are released by immune cells in response to numerous antigens, bacterial polysaccharides and lectins (1). Patients with chronic renal failure commonly present with abnormalities of immune function strictly correlated with abnormalities of immune cell reactivity,

phenotype alternations of receptors and altered expression of cell surface receptors. These abnormalities are caused by impaired excretory function of kidneys and the accumulation of uremic toxins in addition to bioincompatibility of dialyzer membranes (2, 3).

The contact of blood with dialyzer membrane leads to neutropenia and morphological changes of polymorphonuclear leukocytes. Experimental studies demonstrated stimulation and degranulation in these cells after their contact with dialyzer membranes. These abnormalities may likely be associated with the process of "inefficient phagocytosis" elicited by the adherence of polymorphonuclear leukocyte to foreign surface. Further, inflammatory mediators are released in the course of degranulation. Another process engaged in blood and dialyzer membrane interactions is the activation of monocytes leading to the increased release of IL-1, which in turn leads to the release of IL-2 by monocytes (4-6). Numerous research studies on the synthesis and the release of proinflammatory cytokines IL-1 β , IL-2, IL-6, IL-8 and TNF- α in patients with chronic renal failure on maintenance hemodialysis provide contradictory data. Although some of these studies demonstrated increased serum levels of the proinflammatory cytokines prior to and in the course of hemodialysis, other studies indicated that cellular activation and cytokin synthesis is only transient and the increase of the

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Abbreviations: CRF, chronic renal failure; CRP, C-reactive protein; HD, hemodialysis; SD, standard deviation.

Table 1. The study population characteristics

Study parameter	HD patients (n = 15)	Healthy (n = 15)
Age (years)	36.4 \pm 2.9	38.6 \pm 3.5
Gender (M/F)	7/8	8/7
Duration of HD therapy (mths)	68 \pm 10	-
Serum creatinine (μ mol/L)	1050 \pm 95	101 \pm 8.4
Serum urea (mmol/L)	28.2 \pm 3.8	5.2 \pm 3.5
Chronic renal disease		
Glomerulonephritis	6	-
Pyelonephritis	3	-
Hypertension nephropathy	4	-
Polycystic renal disease	2	-

serum levels is rather moderate (6-12).

This study was designed to assess serum levels of a panel of inflammatory cytokines in chronic renal failure patients on regular maintenance hemodialysis before, 20, 60 and 240 minutes of a single HD session.

Materials and Methods

Study population

The study included 15 chronic renal failure patients on regular HD maintenance therapy and 15 healthy controls. The studied population characteristics were presented in Table 1.

The standard four-hour hemodialysis sessions were performed three times a week with Braun-Dialog equipment (Braun, Melsungen, Germany) with cuprophane membranes (Clirans C101; Terumo Corp., Tokyo, Japan). The average Kt/V index in HD patients was 1.19 \pm 0.1 and the protein catabolism index was 1.42 \pm 0.2 g/kg/day. None of the patients suffered from any symptoms of infections. Nor in any of them laboratory evidence of HBs antigenemia, anti-HCV or anti-HIV antibodies were found. They did not receive any medications known to affect immune functions and the time period from the last blood transfusion was not shorter than 6 months. The blood samples were collected before HD sessions directly from arteriovenous fistula, whereas were collected from arterial duct of the dialyzer at 20, 60 and 240 min after the session started. The study protocol was reviewed and approved by the Institutional Review Board of Medical University with respective decision No.231/97.

Determination of cytokine serum levels

The concentration of IL-2 was determined with Quantikine Human Interleukin Immunoassay ELISA test provided by R&D Systems. The test sensitivity was 7 pg/cm³. The concentration of IL-6 and IL-8 was also assessed with Quantikine Human Interleukin Immunoassay (R&D Systems)

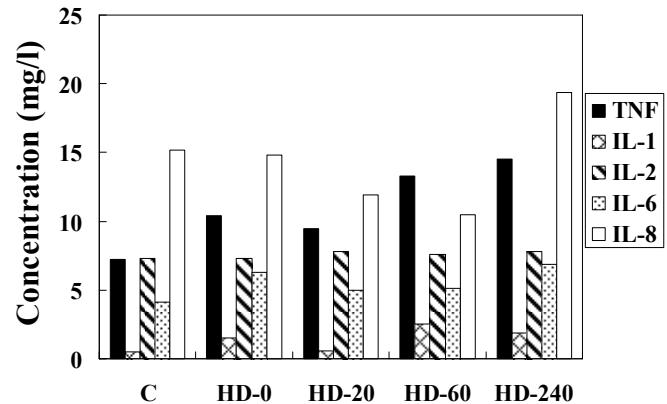


Figure 1. Serum TNF- α , IL-1, IL-2, IL-6 and IL-8 concentration after dialysis maintenance therapy. C, control healthy group; HD, patients on hemodialysis maintenance therapy.

with sensitivity levels of 0.7 pg/cm³ and 10 pg/cm³, respectively. The concentration of TNF- α and IL-1 β was determined with assay kits from Amersham International (Amersham; UK) following the manufacturer's instructions. The test sensitivity in this case was 4.4 pg/ml TNF- α and 0.3 pg/ml in case of IL-1 β .

Statistical analysis

The results are expressed as arithmetic mean (X) and standard deviation (SD). The comparisons between the study groups were performed with sum rank Wilcoxon test, whereas Wilcoxon test was used for the comparisons within a group. The differences were considered significant at $p < 0.05$.

Results

After 20 minutes of HD session with cuprophane membrane, there was a significant decrease of TNF- α concentration, whereas its concentration in blood serum increased and remained at the increased level up to the end of the session. TNF- α concentration was significantly increased in HD patients when compared with the healthy controls (Figure 1). The concentrations of IL-1 β were significantly decreased 20 minutes after HD session started, but it significantly increased 60 minutes later and remained at the increased levels until the end of the session. Serum IL-1 β concentrations were significantly increased in HD patients when compared with healthy group (Figure 1). Serum IL-2 concentrations in HD patients were similar to the levels found in healthy controls and it did not change during single HD session (Figure 1). At each given time points during single HD session, serum concentrations of IL-6 were significantly increased when compared with healthy controls. Further, at 20 and 60 min after the session was begun, serum concentrations of IL-6 were decreased compared with the levels found before HD session, whereas at the end of HD, at

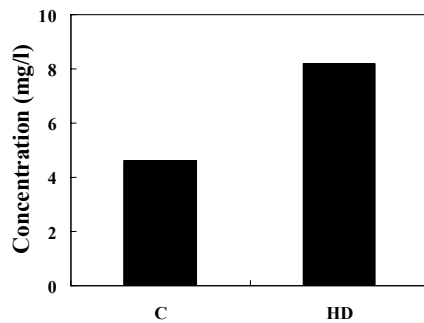


Figure 2. Serum CRP concentration after dialysis maintenance therapy. C, control healthy group; HD, patients on hemodialysis maintenance therapy.

240 min it was increased above the reference level of the healthy controls (Figure 1). The concentrations of IL-8 were lower in HD patients when compared with healthy, and the concentrations were further significantly decreased at 20 and 60 min after the HD session was started. However, IL-8 concentrations were significantly increased within 240 minutes of HD session in comparison with the values found before the session (Figure 1). Further, there was an increase of serum CRP levels in HD patients when compared with that of controls (Figure 2).

Discussion

Cytokines are released in the course of HD session mainly by monocytes, and factors responsible for monocytes activation include endotoxins that may be present in dialysate fluid, activated complement and dialyzer membrane itself (13-15). The physical contact between artificial dialyzer membrane and some compounds in dialysate fluid leads to activation of alternative pathway of complement. The type of dialyzer membrane is crucial in this process (8, 16, 17). In the first minutes of hemodialysis, serum levels of C3a and C5a were rapidly increased and then gradually returned to baseline level, as it is in case of cellulose membranes. The decrease of complement compounds in the initial phase of dialysis leading to augmented inflammatory reaction may explain the decrease of IL-1, IL-6, IL-8 and TNF- α in our study. The membranes with enriched cellulose and synthetic membranes are characterized with lower level of alternative pathway complement activation. The major role in induction of inflammatory response is attributed to C5a, which increases adherence and aggregation of leukocytes and increases generation of reactive oxygen species from these cells in addition to the stimulation of cytokin release from mononuclear cells.

The proinflammatory cytokines are activated not only by complement factors, but also the agents of bacterial origin circulating in dialyzing fluid that are capable of activation of peripheral mononuclear cells releasing numerous cytokines, including interleukin-1 and tumor necrosis factor (9). The dialyzing fluid may contain Gram positive bacteria and

Pseudomonas aeruginosa, inducing the cytokine release with lipopolysaccharide endotoxins and exotoxins, which in spite of high molecular weigh can penetrate through dialyzing membranes and are responsible for the acceleration of inflammatory reactions. The increased levels of inflammatory mediators, including CRP and interleukin-6 are associated with atherogenesis. The progressing chronic renal failure is related with the alternations of serum protein composition and vascular damage. Increased level of homocysteine, lipoprotein a, generation of reactive oxygen species and malnutrition is common in both patients on maintenance hemodialysis and chronic renal failure patients prior to hemodialysis therapy (18). In the current study, the serum levels of CRP in HD patients were increased demonstrating one of the inflammatory responses underlying HD related abnormalities. Monocyte activation and the synthesis of proinflammatory cytokines lead to self propelling reactions of synthesis and release of anti-inflammatory receptors and cytokines. The release of IL-1 β , IL-6 and TNF- α stimulates lymphocytes for synthesis of IL-2 (19). The increase of these cytokines in the course of HD session is not consistently confirmed in various studies. The HD related increase of inflammatory mediators is explained with the activation of macrophages and neutrophils (20-23). There are some reports on lack of relevant changes of serum cytokine levels in HD patients before and after HD session in comparison with healthy subjects that may hypothetically be linked with anemia and the intracellular increase of IL-1 β retained without release to peripheral blood (17). In current study, there was significant increase of serum TNF- α and IL-1 β 20 minutes after HD session with cuprophane membrane started, whereas after 60 minutes of HD session there was an increase of cytokines, which remained until a session was over. The important role of IL-1 β as an inflammatory mediator is associated with pathogenesis of numerous diseases. Monocytes stimulated with endotoxins release IL-1 β and TNF- α , which also play a crucial role in inflammatory and immune reactions, also observed in this study. The stimulated mononuclear cells release not only IL-1 β and TNF- α but also IL-6, which displays both proinflammatory and anti-inflammatory properties. IL-6 is also an endogenous pyrogen, which activates acute phase proteins and suppresses albumin synthesis, enhances proliferation of B and T lymphocytes, contributing to the synthesis of IL-2 (7, 9).

There are reports on the increased serum levels of IL-6 in patients with chronic renal failure and in HD patients (16) as well as the increased spontaneous release of IL-6 and TNF- α by peripheral blood leukocytes in HD patients and the increased release of IL-6 in the course of HD session (19). These reports support our observations on increased IL-6 serum levels in HD patients at each of the studied time points during HD session. After 20 and 60 minutes of HD session, the serum concentrations of IL-6 were decreased when compared with the values prior to the session. However, IL-6 levels after 240 minutes were increased. The concentration of IL-6 in peripheral blood was increased in HD patients compared with healthy controls. IL-8 is released mainly by

stimulated macrophages in addition to fibroblasts, endothelial cells in response to cytokines released by macrophages, mainly IL-1 and TNF- α (6). We found serum concentration of IL-8 in HD patients was decreased, and even more lowered at 20 and 60 min in the course of HD session. However, IL-8 levels were significantly increased at the end of HD session if compared with the values prior to the session. The levels of IL-2 in HD patients were similar to the healthy control values and they did not change during the session.

Some research evidence indicating decreased spontaneous and LPS-elicited cytokine release by leukocytes in HD patients and during HD session may explain common immune defects in HD patients (6, 9). The relation between the degree of monocytes activation and the type of dialyzer membrane was equivocally demonstrated as well as the cytokine release was found to be correlated with the degree of dialyzer membrane bioincompatibility (14).

In conclusion, CRP concentration was increased in HD patients if compared with healthy controls. The concentrations of IL-1, IL-6, IL-8 and TNF- α were increased, whereas the serum level of IL-2 was not altered during a single HD session.

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