EFFECTS OF HCV-RNA POSITIVITY ON SERUM IL-1 BETA LEVELS IN CHRONIC HEMODIALYSIS PATIENTS

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ABSTRACT

Purpose: *Hepatitis C virus (HCV) positivity and inflammatory cytokines such as interleukin(IL)-1 beta, tumor necrosis factor-alpha and IL-6 secreted by activated macrophages are known to be important morbidity factors in chronic hemodialysis (HD) patients. In this preliminary study, we aimed to compare serum IL-1 beta levels of 20 HCV-RNA-positive and 23 HCV-RNA-negative chronic HD patients.* ***Methods:*** *HCV-RNA-positivity and serum IL-1 beta levels were studied using nested reverse transcriptase-polymerase chain reaction and ELISA methods, respectively.* ***Results:*** *We could detect no statistically significant difference between serum IL-1 beta levels in HCV-RNA-positive and HCV-RNA-negative groups ($p>0.05$).* ***Conclusion:*** *To our knowledge, this is the first study to examine the relationship between serum IL-1 beta level and HCV infection in HD patients. We had expected the level of IL-1 beta to be higher in HCV-RNA-positive group, and believe that the blood-dialyzer interaction strongly activated mononuclear cells, thus generating elevated levels of IL-1 beta in both groups. This could explain why HCV infection apparently did not affect serum IL-1 beta levels.*

Key Words: *HCV-RNA, IL-1 Beta, Hemodialysis.*

INTRODUCTION

Interleukin (IL)-1 beta is an important proinflammatory cytokine mainly secreted by monocytes. Elevated serum levels of IL-1 may lead to a variety of symptoms, including fever, hypotension, malnutrition, bone resorption, cartilage erosion (1) frequently seen in chronic hemodialysis (HD) patients (2). Blood-dialyzer interaction is known to activate monocytes and cause increased secretion of IL-1 (3). Furthermore, chronic hepatitis C virus (HCV) infection, which is very common in HD patients, is often associated with a rise in serum IL-1 (4). The effects of chronic HD and HCV infection on various cytokines, especially those secreted by T lymphocytes have been investigated (5). However, there is currently no such information on IL-1 beta. The aim of this preliminary study was to investigate the effect of HCV-RNA-positivity on serum IL-1 beta levels in chronic HD patients.

This study was presented as a poster in the XXXVIIIth Congress of European Renal Association and European Dialysis and Transplant Association (EDTA), in Vienna-Austria, June 24-27, 2001.

MATERIAL AND METHODS

Patients: Serum samples from 20 HCV-RNA-positive (11 females of mean age ±SD:49 ±12 years and 9 males of mean age ±SD:47±16 years) and 23 HCV-RNA-negative (control group; 12 females of mean age ±SD:53±13 years and 11 males of mean age ±SD:46±19 years) HD patients were studied. The groups were randomly selected from volunteers who were on chronic HD treatment at Başkent University Hospital. Mean age and gender distribution were comparable in both groups. The etiologies of chronic renal failure in both groups were nephrolithiasis, diabetes mellitus, glomerulonephritis (GN), and polycystic kidney disease. There was no statistically significant difference between the two groups regarding the distribution of these etiologies.

Serum samples: Peripheral venous blood samples from all patients were centrifuged at 1600 g for 10 min to separate off the sera. Serum samples for HCV-RNA extraction were analyzed immediately while those for IL-1 beta level measurement were kept at -20°C until the day they were assessed.

HCV-RNA testing: Serum samples were investigated for the presence of HCV-RNA using nested reverse transcriptase-polymerase chain reaction (RT-PCR) analysis. HCV-RNA extraction was performed using the acid guanidium thiocyanate-phenol-chloroform method (6). Extracted RNA was kept at -80°C until the day the RT-PCR was run. For amplification of the 5' non-coding region of HCV, we used the primer sets 209/940 (external set) and 211/939 (internal set). The RT-PCR was performed in a commercially available system (Calypso RT-PCR System, DNA Amp Ltd., UK) according to the manufacturer's instructions. Amplification products of 251 base pairs were run in 2% agarose gel electrophoresis and analyzed under a UV transilluminator.

IL-1 beta level measurement: Serum IL-1 beta levels were measured using a commercially available ELISA kit (Cytelisa, Human IL-1 beta, Cytimmune Sciences Inc., Maryland, USA) according to the manufacturer's instructions. Sensitivity of the assay was 0.87 pg/ml. Optical densities (OD) were read at 490 nm. All samples, standards and blank were studied in duplicate and the arithmetical mean of each pair of OD's was recorded. Serum IL-1 beta concentrations were calculated in pg/ml using regression-correlation analysis of Microstat statistical software according to OD values and concentrations of the standards provided with the kit ($p=0.039$, $r=0.897$).

Statistical analysis: Student's t-test and Mann Whitney U-test were performed for statistical analysis. Results were expressed as mean±SD. $p<0.05$ was considered statistically significant.

RESULTS

The calculated serum IL-1 beta levels of HCV-RNA-positive and HCV-RNA-negative (control) groups were 391.8±179 pg/ml and 409.7±179 pg/ml, respectively. There was no statistically significant difference between two groups ($p=0.97$). We also categorized and compared the data on the basis of sex (Fig. 1). This revealed no statistically significant difference between serum IL-1 beta levels of HCV-RNA-positive and -negative females (393.9±180 pg/ml and 390.7±193 pg/ml, respectively; $p=0.805$). The same held true for HCV-RNA-positive and -negative males, whose corresponding serum IL-1 beta levels were also statistically similar (389.2±188 pg/ml and 430.5±170 pg/ml, respectively; $p=0.79$).

DISCUSSION

Monocytic activation with associated cytokine production is a well-known event in the course of dialysis treatment (7). Several studies have reported increased cytokine production secondary to blood interaction with bioincompatible dialysis components, or as a result of blood-dialyzer interaction during HD. IL-1, tumor necrosis factor(TNF)-alpha and IL-6 are the three main proinflammatory cytokines involved in the pathogenesis of HD-related diseases (2, 3). The interleukin hypothesis suggests that the release of these cytokines acts as an underlying pathophysiologic event in HD-related acute manifestations such as fever and hypotension. Furthermore, cytokine overproduction may alter sleep patterns, nutritional status and bone remodeling, and may have a long-term effect on mortality of uremic patients by altering the immune response and increasing susceptibility to infection (2).

Rousseau et al. investigated the effects of different types of hemodialytic membranes (polysulfone, cellulose acetate, cuprophane) on cytokine production by circulating neutrophils (8). They found that the percentage of leukocytes expressing IL-1, IL-1ra, TNF-alpha and IL-8 was increased in HD patients subjected to HD with cuprophane membranes. Kimmel et al. (9) hypothesized that higher levels of proinflammatory cytokines would be associated with higher mortality. They found that the mean levels of circulating cytokines (IL-1, IL-2, IL-4, IL-5, IL-6, IL-12, IL-13 and TNF-alpha) in HD patients were higher compared to controls. They detected no difference in cytokine levels in HD patients treated with different membranes types. In our study, cuprophane dialyzer membranes were used in all patients, therefore membrane type was not a confounding variable.

Chronic HCV infection is associated with significant morbidity and mortality (10), and this virus is the most frequent cause of liver disease in dialysis and transplant recipients (11, 12). Approximately 20%-30% of the HD population is infected with HCV (12). HCV infection itself may be involved in the pathogenesis of GN. Alternatively, GN or severe renal insufficiency may increase the probability of HCV infection (13).

A number of studies have documented elevated levels of serum IL-1 beta, IL-2r, IL-6, and TNF-alpha levels were found to be elevated in patients with chronic HCV infection (4, 14, 15). It has also been proposed that imbalance of Th1 (e.g. IL-2, interferon(IFN)-gamma) and Th2 (e.g. IL-4 and IL-10) cytokine production plays a role in the immunopathogenesis of persistent HCV infection (16). Levels of circulating IL-2, IL-4, IL-10 have been shown to be significantly higher in HCV-positive patients compared to healthy controls (17) while IFN-gamma level was higher (16) or unchanged (17). On the basis of these data, we expected to find higher serum levels of IL-1 beta in the HCV-RNA-positive HD group than the HCV-RNA-negative HD group.

As mentioned above, both blood-dialyzer interaction and chronic HCV infection are important morbidity factors in HD patients, and these may have important influences on cytokine levels. Martin et al. (5) investigated the cytokine responses related to HD and HCV infection in chronic renal disease. They found that the altered cytokine response mainly involved the Th1 lymphocyte phenotype (thus, production of IFN-gamma, etc.), whereas stimulation of IL-6 and IL-10 (Th2 phenotype cytokines) was not impaired. The pattern of response was similar in HD patients with and without HCV infection, but IL-10 stimulation was higher in HCV-positive HD patients. Overall, their study revealed markedly abnormal general and HCV-infection-related cytokine responses in HD patients.

To our knowledge, no previous study has examined the relationship between IL-1 beta and HCV infection in HD patients. Monocytes are the main source of secreted IL-1 beta in humans. IL-1 beta is an important factor in the effector phase of inflammatory and immune responses, plays a central role in the host defense and acts as a pyrogen. IL-1 induces secretion of IL-6, IL-8, granulocyte-colony stimulating factor and TNF-alpha, and also activates bone resorption, induces cartilage breakdown as well as the secretion of acute phase proteins from hepatocytes (1).

Blood-dialyzer interaction (3) and HCV infection (4, 14, 15) are known to increase serum IL-1 beta levels. As explained, the use of cuprophane for all patients in our study eliminated the possibility of IL-1 beta alterations related to membrane type. With this potential

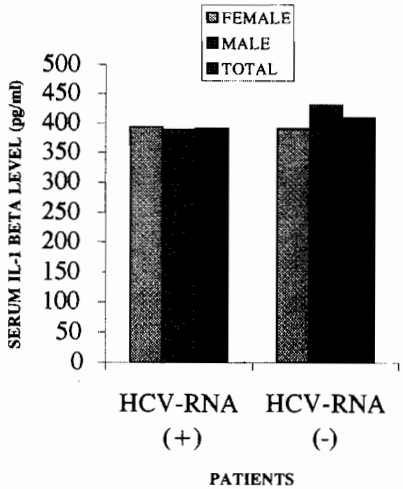

Fig. 1: Serum IL-1 beta levels of HCV-RNA-positive and HCV-RNA-negative hemodialysis patients. There was no statistically significant difference between HCV-RNA-positive and HCV-RNA-negative groups (total and on the basis of sex) for serum IL-1 beta levels ($p>0.05$ for all).

problem removed, we hypothesized that serum IL-1 beta levels would be higher in the HCV-RNA-positive HD patients than in their HCV-RNA-negative counterparts. However, the group mean levels were similar, (391.8±179 pg/ml vs. 409.7±179 pg/ml ($p>0.05$)). We also found no statistically significant differences when groups were divided according to sex. For each sex, the IL-1 beta levels in the HCV-RNA-positive group were comparable to those in the HCV-RNA-negative group ($p>0.05$ for both) (Fig. 1).

According to our results, serum IL-1 beta levels appear to be much more strongly influenced by blood-dialyzer interaction than by HCV infection. However, this is a preliminary study, and the investigation needs to be repeated in a larger group of patients. Also, an in vitro study with isolated blood monocytes would be valuable as this would allow more definitive conclusion to be made about the links between IL-1 beta response, HD, and HCV infection in chronic renal disease patients.

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