

## Relationship among MIF, MCP-1, viral loads, and HBs Ag levels in chronic hepatitis B patients

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**Background/aim:** To determine whether macrophage migration inhibitory factor (MIF) and monocyte chemoattractant protein-1 (MCP-1) levels in patients with hepatitis B (HB) are different than in normal individuals and whether the HB surface antigen (HBs Ag) level and viral load are correlated with each other and with the two aforementioned parameters.

**Materials and methods:** Sera were obtained from 52 chronic active HB (CAHB) patients and 33 healthy controls, and their MIF and MCP-1 levels were measured. Statistical analyses were performed. A value of  $P < 0.05$  was considered statistically significant.

**Results:** The MIF and MCP-1 values of the control group were increased compared to those of the CAHB group. The MIF and MCP-1 levels were negatively correlated with HBs Ag levels and viral loads. The MIF and MCP-1 levels were positively correlated. The HBs Ag levels and the log<sub>10</sub> of the viral loads were positively correlated.

**Conclusion:** We conclude that the negative correlation of MIF and MCP-1 with viral load and HBs Ag levels may be due to T-cell deficiency, antinuclear antibody seropositivity, and/or inhibition of chemokine ligand 2 receptors by viral antigens. More studies with a greater number of subjects are needed to evaluate the potential role of MIF and MCP in CAHB.

**Key words:** Chronic active hepatitis B, macrophage migration inhibitory factor, monocyte chemoattractant protein-1, viral load, HBs Ag

### 1. Introduction

Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine that activates monocytes, macrophages, neutrophils, and T cells and promotes the synthesis of cytokines, including tumor necrosis factor- $\alpha$ , interleukin (IL)-1 $\beta$ , IL-6, and IL-8 (1-3). MIF is released from an intracellular pool of monocytes/macrophages in response to various pathological stimuli, including inflammation and infection (4-6). High MIF values were found to be correlated with the severity of disease in various infectious diseases, such as pyelonephritis (7), dengue fever (8), and hepatitis B (HB) (9).

The monocyte chemoattractant protein-1 (MCP-1) is secreted from macrophages, monocytes, endothelial cells, epithelial cells, and fibroblasts after stimulation

with microbial products or cytokines (10,11). It primarily attracts monocytes and T cells (12). In earlier studies, MCP-1 contributed to the activity of neutrophils during severe bacterial infections (13,14). The administration of MCP-1 to animals also provided protection from lethal doses of *Pseudomonas aeruginosa* and *Salmonella typhimurium* (15).

In this study, we investigated whether MIF and MCP-1 levels in patients with chronic active hepatitis B (CAHB) are different from those of normal individuals and whether HB surface antigen (HBs Ag) levels and viral loads were correlated with each other and the aforementioned parameters. Thus, we aimed to investigate whether there is a relationship between stage of disease and the level of these two tests in CAHB patients.

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## 2. Materials and methods

This study was performed with the approval of the Noninvasive Clinical Research Ethics Committee of the Namık Kemal University Medical Faculty and with the consent of the participating patients. HBs Ag levels in the sera samples were measured using the Vitros ECiQ diagnostic system (Johnson & Johnson, USA), and viral loads were measured using a fluorine detection system (İontek, Turkey) using real-time PCR according to the manufacturer's recommendations. Sera were obtained from 52 patients with CAHB patients and 33 healthy controls whose hepatic enzymes, acute phase reactants, and biochemical parameters were in normal ranges and who had no liver or acute/chronic inflammatory disease. Their MIF and MCP-1 levels were measured using commercially available ELISA kits (MIF: Hangzhou Eastbiopharm Co. Ltd., China; MCP-1: Sunred Biological Technology, China) according to the manufacturer's instructions. Statistical analyses were performed using SPSS 18.0 for Windows (SPSS, Inc., USA). Multiple group differences were compared using an ANOVA test, and Pearson's correlation coefficients were used to analyze the correlations between the MIF, MCP-1, and HBs Ag levels and log<sub>10</sub> of the viral load. A value of  $P < 0.05$  was considered statistically significant.

## 3. Results

Fifty-two chronic HB patients and 33 controls were enrolled in our study. The MIF and MCP-1 values of the control group were increased compared to those of the CAHB group ( $3.08 \pm 1.23$  ng/mL versus  $2.541 \pm 72$  ng/mL [ $P = 0.01$ ] and  $66.69 \pm 63.23$  ng/mL versus  $36.01 \pm 29.74$  ng/mL [ $P = 0.001$ ]). The results of the Pearson's correlation coefficient analysis showed that the MIF and MCP-1 levels were negatively correlated with the HBs Ag levels ( $P = 0.006$  and  $P = 0.002$ , respectively) and with the log<sub>10</sub> of the viral loads ( $P = 0.000$  and  $P = 0.000$ , respectively). The MIF and MCP-1 levels were positively correlated ( $P = 0.000$ ), and the HBs Ag and the log<sub>10</sub> of viral loads were positively correlated ( $P = 0.004$ ).

## 4. Discussion

As expected, the log<sub>10</sub> values of the viral load and the HBs Ag levels were positively correlated. The MIF values were negatively correlated with both parameters mentioned and they were significantly lower in the CAHB group than in the control group. Higher MIF values have been detected in individuals with bacterial and viral infections compared to healthy individuals. Studies of mice models of *Escherichia coli* peritonitis or endotoxic shock revealed increased serum MIF values, and they showed that neutralization of MIF with antibodies protected the mice from lethal shock and sepsis (16,17). In another mouse

model of exotoxin-induced shock, it was reported that streptococcal and staphylococcal exotoxin induced MIF secretion in macrophages and that administration of anti-MIF antibodies increased survival (18). These findings indicate that MIF has an important role in bacterial infections. Several studies have shown that septic patients with high serum MIF levels appear to have an elevated risk of mortality compared to patients with lower levels (19–21). In some diseases characterized by inflammation and in some viral diseases, MIF values were higher than in normal individuals (22,23). MIF values in patients with chronic hepatitis B were higher than in healthy control groups in a study by Zhang et al. (9). In another study, no statistically significant difference has been observed between the MIF levels of patients during the immune tolerance period and normal controls. (24). Lower findings in our results may be due to some metabolic events that prevent the rise in MIF values, or cytokine releases. However, the MIF values of the CAHB patients in the present study were lower than those of the healthy controls. This finding may be related to T-cell deficiency. According to the literature, immunocompetent adults who fail to clear acute HBV show reduced CD4+ T-cell and cytotoxic T-lymphocyte responses. In one study, peripheral CD4+ T-cell and cytotoxic T-lymphocyte responses to chronic infection were weak and narrowly focused (25). Various studies in the literature have confirmed the findings of the present study. For example, Kim et al. (26) observed a decrease in MIF secretion in macrophages in response to treatment with hypertonic saline. Choi et al. (27) observed a decrease in MIF expression in T cells treated with hypertonic saline associated with T-cell dysfunction.

In studies conducted with parvovirus B19-dengue virus (28) and HIV-infected patients (29,30), MCP-1 levels were positively correlated with the viral loads. In studies of the influenza virus, anti-MCP-1 treatment significantly reduced the infiltration of macrophages into the lungs, and blocking MCP-1 reduced the neutrophil population in bronchoalveolar lavage (31). Increased viral loads were observed in MCP-1 knockout mice, with reduced leukocyte recruitment to the infected lungs (32). Our results are consistent with the findings of these studies. In a previous study, no statistically significant difference was observed in MCP-1 values of chronic hepatitis B patients among 4 periods (period during which ALT was not 2 times higher than the normal but before the period of HBV DNA fluctuations, peak period of ALT, peak period of HBV DNA, and period after acute inflammation) (33). However, no study comparing MCP-1 levels of chronic hepatitis B patients with a healthy control group has been reported in the literature. In our study, MCP-1 levels in patients with chronic hepatitis B were lower compared to the control group. Different factors may be responsible for

the impairment of MCP-1. In one study, positive antinuclear antibody (ANA) values in patients showed a negative correlation with MCP-1 levels (34). ANA values may also explain the findings of the present study. However, as we did not collect data on the ANA values of the patients, we cannot draw any definite conclusions. In other studies, researchers observed that when the viral load increased, numerous viral proteins antagonized chemokines by competitively binding their receptors, such as CCR5 and CXCR4 (35–38). They suggested that levels of chemokines might be correspondingly downregulated (39). As we did not evaluate CCL-2 receptors, we cannot draw any conclusions on this matter. The absence of data on ANA levels and CCL-2 receptors are limitations of the present study.

In conclusion, MIF and MCP-1 levels were negatively correlated with the viral load and HBs Ag levels in our

study. We infer that this may be due to T-cell deficiency, ANA seropositivity, and/or inhibition of CCL-2 receptors by viral antigens. More studies with a greater number of subjects are needed to determine the potential impact of the aforementioned factors on MIF and MCP-1 levels. For evaluation of significance of MIF and MCP-1 levels in the clinical diagnosis, a large-scale study with a greater number of samples and other factors (cytokines, receptors, etc.) is needed.

A low level of MIF and MCP-1 at the beginning of the disease may have led to chronicity. However, to demonstrate the relationship between these two tests and the pathogenesis of chronic hepatitis, in addition to the tests used in management of patients with acute hepatitis, MIF and MCP-1 scores have to be considered in future studies.

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