Evaluation of Interleukin-10 Levels in Patients Diagnosed with Chronic Hepatitis

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ABSTRACT

Objective: *One of the most important factors playing a role in chronic hepatitis B pathogenesis is cytokine release and one of the cytokines with anti-inflammatory characteristic is interleukin-10 (IL-10). The aim of the present study is to examine IL-10 levels in patients with chronic hepatitis B.*

Subjects and Methods: *Sixty-three patients with chronic hepatitis B disease who had not received any antiviral treatment were included in the study. Serum IL-10 level was investigated by enzyme-linked immunosorbent assay (ELISA) method. In the control group, 25 healthy individuals with mean age similar to the patient population were included. Control and patient groups were compared and data were statistically analysed.*

Results: *Interleukin-10 levels of 25 patients with hepatitis B virus (HBV) DNA levels between 2000 and 20 000 IU/mL were compared with those of 25 subjects in the control group, and the level in the chronic hepatitis B group was statistically significantly higher (*$p < 0.05$*). Interleukin-10 levels of 38 patients with HBV DNA > 20 000 IU/mL were statistically significantly higher than those in the control group. When chronic hepatitis B patients were compared among themselves, IL-10 levels increased as HBV DNA levels increased. Also, when IL-10 levels of hepatitis B 'e' antigen (HBeAg) positive patients were compared with those of HBeAg negative patients, the difference was not statistically significant.*

Conclusion: *It is believed that decreasing IL-10 levels by various methods would have significant contributions in disease progression and treatment. Moreover, IL-10 level may be an important marker in HBeAg seroconversion and evaluation of treatment response.*

Keywords: Chronic hepatitis B, cytokine, interleukin-10

Evaluación de los Niveles de Interleuquina-10 en Pacientes Diagnosticados con Hepatitis Crónica

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RESUMEN

Objetivo: *Uno de los factores más importantes que desempeña un papel en la patogénesis de la hepatitis B crónica es la liberación de citoquinas, y una de las citoquinas con característica antiinflamatoria es la interleuquina-10 (IL-10). El objetivo del presente estudio fue examinar los niveles de IL-10 en pacientes con hepatitis B crónica.*

Sujetos y métodos: *Sesenta y tres pacientes con la enfermedad de la hepatitis crónica B, que no habían recibido ningún tratamiento antiviral, se incluyeron en el estudio. Mediante el método del ensayo inmunoenzimático ligado a enzimas (ELISA), se investigó el nivel de IL-10 en suero En el grupo control, se incluyeron los 25 individuos sanos con edad promedio similar a la población de pacientes. Se compararon los grupos de control y pacientes, y los datos fueron analizados estadísticamente.*

Resultados: *Los niveles de interleuquina-10 de 25 pacientes con niveles de ADN del virus de la hepatitis B (VHB) entre 2000 y 20 000 UI/mL fueron comparados con los de 25 sujetos del grupo control, y el nivel en el grupo de la hepatitis B crónica fue estadísticamente significativamente superior (*$p < 0.05$*). Los niveles de interleuquina-10 de 38 pacientes con ADN VHB > 20 000 UI/mL fueron significativamente superiores a los del grupo control. Al compararse los pacientes de hepatitis B crónica entre sí, se observó que los niveles de IL-10 aumentaban a la par con el aumento de los niveles de ADN del VHB.*

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DOI: 10.7727/wimj.2014.154

Por otra parte, cuando los niveles de IL-10 de pacientes con antígeno 'e' (HBeAg) positivo de la hepatitis B fueron comparados con los de los pacientes HBeAg negativo, la diferencia no fue estadísticamente significativa.

Conclusión: *Se cree que la disminución de los niveles de IL-10 mediante varios métodos, contribuiría significativamente a la progresión de la enfermedad y al tratamiento. Por otra parte, el nivel de IL-10 puede ser un marcador importante de la seroconversión de HBeAg y la evaluación de respuesta al tratamiento.*

Palabras claves: Hepatitis B crónica, citoquinas, interleuquina-10

West Indian Med J 2015; 64 (1): 72

INTRODUCTION

Hepatitis B virus (HBV) infection is one of the important chronic viral infections. There are approximately 400 million HBV carriers in the world; complications such as fulminant hepatic failure, cirrhosis and hepatocellular carcinoma develop annually in 250 000 of them. While the majority of adult infections remit, chronicity develops in 5−10% of cases (1).

Adaptive immune response, which develops against HBV (a hepatotropic non-cytopathic virus) plays a key role in infection control. After the virus is taken into the hepatic cells, infection ensues and host response is initiated. The virus in the hepatic cell is processed by dendritic cells (DC), and it is presented to the immune system cells as an antigen (2).

Dendritic cells in the liver recognize viral particles by Toll-like receptors (TLRs). Dendritic cells process viral antigens, and present them to immune system cells through the major histocompatibility complex (MHC)-1 and MHC-2. Some cytokines are secreted by activation of these cells, and immunologic response is designed according to them (2−4).

Cytokines are small soluble proteins secreted by the immune system and other body cells. They act as a part of intercellular interaction in the immune system. These proteins are bound to their specific cellular receptors; they function by autocrine or paracrine effects and may inhibit or induce cytokine regulating genes. Until now, 100 different cytokines have been reported, and they are grouped according to their roles. These proteins play a key role in directing, polarization, and regulation of the immune response. Antigenic stimulation, defining immune response development, is brought on by a combination of cytokines (2−4).

The most important cytokine among them is interleukin-10 (IL-10), which has the old name cytokine synthesis inhibitor factor (CSIF). Interleukin-10 was first defined in 1989 as the cytokine synthesis inhibitor. It is made up of 178 amino acids and has the molecular weight of 36 kDa. In human beings, IL-10 is synthesized mainly by monocytes, T cells, B cells, macrophages and DCs (5−7). As a direct effect, it decreases MHC-2 molecules acting on macrophage monocyte functions, and also decreases surface release of CD80/CD86 which are helper stimulators of them (7).

The present study investigated whether there was a correlation between anti-inflammatory cytokine IL-10 level and chronicity in chronic hepatitis patients.

SUBJECTS AND METHODS

Sixty-three patients, who received no antiviral treatment, had chronic hepatitis B disease and attended the Department of Infectious Diseases and Clinical Microbiology of Medical School Hospital at Firat University between June 2010 and May 2011, were included in the study. Twenty-five healthy controls with similar mean age to the patient population were also included. The study was approved by the Ethics Review Committee of the Medical School of Firat University.

Hepatitis B virus DNA levels were studied with Roche Cobas Taqman (COBAS® AmpliPrep/COBAS® TaqMan® HBV Test, v2.0, Roche Diagnostics, Basel, Switzerland) and High Pure PCR Template Preparation Kit was used for hepatitis B virus DNA extraction (Roche, Germany).

Five millilitres of blood samples from patients who are applicable for operation were taken into biochemistry tubes and they were centrifuged for five minutes at 3000 rpm. After separation, sera were stored at -80 ˚C until the day of measurement. Interleukin-10 levels were measured from the obtained blood samples.

Additionally, patient age, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels, hepatitis B 'e' antigen (HBeAg) and anti-HBe states, HBV DNA levels, histological activity indices and fibrosis scores in liver biopsies, and ultrasound findings were recorded for all patients. All biochemical, serological tests and HBV DNA levels were investigated at the central laboratory of Firat University Hospital. Blood biochemistry tests were performed in an Olympus AU2700 device by using enzymatic kinetic method; HBeAg and anti-HBe levels were investigated in an Architect 2000 device by using macro-enzyme-linked immunosorbent assay (ELISA) [Architect System Abbott Diagnostics, Germany].

Serum IL-10 levels were measured by standard ELISA kits (BOSTER, Wuhan, China), using ELISA method and BIOTEK washer (ELX 50TM Microplate, 40710000, Winooski, USA) and BIOTEK reader (ELX 800TM 733310000, Winooski, USA).

Data were entered by using SPSS 12 programme. Mann Whitney U and Kruskal Willis tests were used in statistical significance evaluation.

RESULTS

Interleukin-10 levels in the control group were compared with those in the patient group. In the second group, there were 25

patients with HBV DNA level of 2000 IU/mL–20 000 IU/mL. In the third group, there were 38 patients with HBV DNA level of > 20 000 IU/mL. When the control group and the second group were evaluated using Mann Whitney U test, it was determined that high IL-10 level was statistically significant ($p = 0.01$). Statistical difference was also determined in IL-10 level between the third group and the control group ($p < 0.05$). When the two groups with high HBV DNA levels were compared, IL-10 level was higher in the third group, but the difference was not statistically significant ($p > 0.48$). When IL-10 levels in all groups with HBeAg positive and negative patients were evaluated, IL-10 level was detected lower in the HBeAg positive group, whereas higher in the HBeAg negative group. However, the differences were not statistically significant ($p > 0.98$).

Patients with high and normal ALT values were evaluated in all groups using Kruskal Willis test. Interleukin-10 levels were low in patients with high ALT levels, but the difference was not statistically significant ($p > 0.567$). Interleukin-10 levels were lower in patients with high histology activity index (HAI) and fibrosis scores, whereas they were higher in patients with low HAI and fibrosis scores. The difference was not statistically significant ($p > 0.366$). The *p*-values of the comparison groups are presented in the Table.

Table: Interleukin-10 levels and corresponding *p*-values when compared between groups

Comparison of groups	***p*-value**
Comparison of first and second groups	0.01
Comparison of first and third groups	0.05
Comparison of second and third groups	0.48
Comparison of normal ALT and high ALT groups	0.56
Comparison of high HAI and grade of fibrosis and low HAI and grade of fibrosis groups	0.36
Comparison of HBeAg positivity and HBeAg negativity	0.98

P-value is significant at $p < 0.05$. ALT – alanine aminotransferase; HAI – histology activity index; HBeAg – hepatitis B "e" antigen

DISCUSSION

Previously, IL-10 was known as an inhibitor of cytokine synthesis, and it was first defined as a Th2 cytokine produced by CD4 cells. It was also reported that IL-10 might affect Th1 response indirectly. Currently, it is known that many cells such as dendritic cells, B cells, macrophages, CD4 T cells, CD8 T cells, NK cells, adaptive and regulatory T cells can also synthesize IL-10. Moreover, IL-10 can also be produced in various liver cell types such as hepatocytes, sinusoidal endothelial cells, Kuppfer cells, hepatic stellate cells and lymphocytes associated with the liver (8).

Interleukin-10 inhibits T cell activity, which is developed against T cell response and viral infections (9, 10). It shows its effects by decreasing molecular expression of antigen presenting cells, and so by decreasing cytokine production, it may prevent T cell activation by interrupting T cell maturation (11). In addition to these indirect effects, IL-10 may directly affect T cells by limiting proliferation, functional differentiation, and their activities (12−16).

Despite negative regulatory functions, it has been shown that IL-10 can stimulate proliferation of NK cells, CD8 T cells, B cells and antibody production (17, 18). Besides, IL-10, which is generally an immunosuppressive, can have effects on pathogen specific immune response through various mechanisms. In persistent viral infections, it has been determined that many cell types produce IL-10 (19, 20). Additionally, suppression states during persistent infections occur because IL-10 limits various immune parameters, so the infection cannot be cleared. Modulation of cells expressing IL-10, which inhibits antiviral activity, is biologically and therapeutically important (21).

Interleukin-10 shows anti-inflammatory activity by inhibiting cytokines secreted from active macrophages such as IL-6, IL-8, IL-12 and tumour necrosis factor-alpha (TNF-α) and interferon gamma (IFN-γ) synthesized from T cells. Moreover, intrahepatic NK cell functions may also be down-regulated by IL-10 secreted from hepatic Kuppfer cells. Intrahepatic NK cell functions may be down-regulated by the immunosuppressive cytokine IL-10 which is secreted from Kuppfer cells. Interleukin-10 also has antifibrinogenic properties (22, 23).

In many studies, it was shown that IL-10 was important in sensitivity to inflammatory diseases, in response to HBs antigen vaccination, in HBV carriers, in HBeAg seroconversion, and in hepatocellular carcinoma related to HBV. Its production can be regulated at transcriptional, post-transcriptional and translational levels (24−26).

In addition to HBV and HCV infections, IL-10 is a cytokine with a key role in regulation of cellular immune response against Ebstein-Barr virus, herpes simplex virus and HIV. Inappropriate release of cytokines such as IL-10 in chronic HCV infected patients was correlated with HCV clearance, fibrogenesis and treatment resistance to interferon (24−26).

In many studies, it was determined that control of cytokine polymorphism has an important role in HBV infection outcomes. The immunological and virological basis of viral persistence has not been well understood in chronic hepatitis B. However, cytokine insufficiency, T cell unresponsiveness, anergy, and viral mutations have been accepted as possible mechanisms (27). The correlation between IL-10, which is produced by HBV antigen stimulated (HBcAg, HBsAg, preS1Ag) peripheral blood monocytic cells (PBMC), and chronic hepatitis B disease activity has not been understood yet. However, it was observed in previous studies that HBV antigens could stimulate IL-10 production (28). It was shown that IL-10 secretion from monocytes was increased by pro-inflammatory cytokine IFN-γ; increased secretion of IL-10 might suppress immune response in HBV infection, and it prevented serious liver damage (29). Additionally, it is known that ex-

cessive IL-10 production may cause viral persistence and weak immune responses.

Hepatitis B core antigen (HBcAg) may cause excessive increase in the immunostimulant IFN-γ; so immunosuppressive IL-10 production is stimulated as a response. It has been shown that IL-10 secretion was stimulated from peripheral blood T cells, monocytes and B cells in chronic hepatitis B patients. Approximately 0−34% of T cells, 2−5% of monocytes and 0−12% of B cells secrete IL-10 against 10 mg/mL HBcAg. It was also shown that monocytes produced 62−70%, T cells produced 26−35% and B cells produced less than 1% of IL-10 in response to HBcAg in PBMC cells (28).

Additionally, it was shown that PBMC stimulation induced against HBcAg induced IL-10 producing cells in healthy controls, and acute HBV limited itself generally in healthy adults by a potent immune response. It was also shown that IL-10 production by PBMC, which was stimulated by HBcAg or HBeAg, had unfavourable effects on treatment response in patients with chronic hepatitis B. Hepatitis B core antigen stimulated IFN-γ inducing Th1 cell, which was increased before hepatitis B exacerbations, and was decreased after the peak (30).

In a study, it was determined that CD4 and CD8+ T cells and monocytes were stimulated by HBcAg and IL-10. It was reported that IL-10 produced by this stimulation induced anergic CD4 and CD8 cells. CD8+ T cells secreting IL-10 are anergic and non-cytolytic, and they inhibit cytolytic T cell proliferation secreting Type 1 cytokine (31). Prezzi *et al* (32) reported that IL-10 secreting CD8+ T cells suppressed tissue damage caused by Type 1 cytokine secreting T cells.

It was observed that HBcAg stimulated IL-10 levels of patients with high ALT levels and detectable DNA was higher than those in patients with normal ALT and detectable HBV DNA (33).

It is known that T cell immune response is correlated with fibrosis and hepatic inflammation in patients with HBV infection and cirrhosis. Interleukin-10 is correlated with HBeAg state, viral replication and progression of liver disease. In another study, the correlation was determined between histological liver damage and IL-10 levels in HBeAg negative chronic hepatitis B patients. In the present study, it was shown that the degree of necroinflammation and IL-10, IFN-γ cytokine levels were correlated in HBeAg negative patients. Moreover, when IL-10 and TNF-α levels were compared between HBeAg negative and positive groups, it was correlated with significant fibrosis (34, 35). In recent studies, IL-10 level has been shown to be a predictor for HBeAg seroconversion in chronic hepatitis B patients (36). In our study, IL-10 levels in the HBeAg positive patients were significantly lower than those in the HBeAg negative group. Our results also indicated that IL-10 levels were being increased for HBeAg seroconversion.

Das *et al* showed that IL-10 levels were correlated with spontaneous exacerbations of liver disease, and IL-10 levels were increased with viral load increase and the peak of liver damage (37). In our study, IL-10 levels were increased with increased viral load.

In another study, it was reported that IL-1β, IL-6 and IL-10 levels were significantly higher in patients with liver cirrhosis than patients diagnosed with chronic hepatitis B. This might be an important marker in progression of chronic hepatitis B into liver cirrhosis (38).

In a study conducted on patients with occult HBV infection, IL-10 levels of 352 patients were determined, and it was found that the levels were significantly higher when compared to control patients (39).

Park *et al* reported that IL-6, IL-8, IL-10 and TNF-α levels were significantly higher in chronic hepatitis B patients resistant to lamivudine than the responsive ones, and suggested that it might be an indicator in monitorization of treatment response in chronic hepatitis B patients (40).

In a national study, it was reported that IL-10 levels in both asymptomatic carriers and chronic hepatitis B patients were significantly higher than those in the control group. However, no significant difference was determined when asymptomatic carriers and chronic hepatitis B patients were compared (41). In another similar study, IL-10 levels were determined significantly higher in chronic hepatitis B patients when compared with inactive carriers (42).

In our study, IL-10 levels were statistically significantly higher in chronic hepatitis B patients when compared with the control group. The positive correlation was detected between high levels of HBV DNA and IL-10, but it was not statistically significant. Our results are consistent with the literature.

In conclusion, IL-10 is an immunosuppressive cytokine. High levels of IL-10 in chronic hepatitis B patients indicate that IL-10 level may be the cause of ineffective immune response. Our results indicate that decreasing IL-10 levels by using various methods may have important contributions in progression and treatment of the disease. Moreover, we believe that IL-10 levels may be an important marker for HBeAg seroconversion and evaluation of the treatment response. Further studies are required on this topic.

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