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Role of tumor necrosis factor- α , interleukin-1 β , interleukin-6 in liver inflammation in chronic hepatitis B and chronic hepatitis C

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ABSTRACT

Aims: Cytokines play important roles in the immunopathogenesis of chronic hepatitis B (CHB) and chronic hepatitis C (CHC) infections. The aim of this study was to examine the changes in serum levels of interleukin (IL)-1 β , IL-6 and tumor necrosis factor (TNF)- α cytokines in patients with CHB and CHC, and the relationship of these cytokines with chronic inflammation, disease progression and fibrosis.

Methods: We prospectively enrolled patients with CHB and CHC and control subjects from August 2016 to August 2018. Liver biopsy samples were obtained as a part of the routine care. Serum levels of IL-6, IL-1 β , and TNF- α were determined by the enzyme-linked immunosorbent assay method.

Results: The final sample included 90 patients with CHB (age, mean \pm SD: 42.6 \pm 11.8) 40 patients with CHC (age, mean \pm SD: 45.1 \pm 13.6) and 50 controls (age, mean \pm SD: 39 \pm 15.0). IL-1 β , TNF- α and IL-6 serum levels were statistically significantly higher in patients with CHB and CHC than in the control group [IL-1 β (ng/mL): 133.7 \pm 37.0, 125.92 \pm 12.7, 85.7 \pm 9.8; TNF- α (ng/mL): 307.9 \pm 68.9, 286.0 \pm 43.2, 72.0 \pm 14.01; IL-6 (ng/mL): 50.6 \pm 10.1, 55.07 \pm 9.79, 8.85 \pm 7.07, respectively, p=0.001]. TNF- α level was statistically significantly higher in patients with significant fibrosis (320.5 \pm 36.9) than those with mild fibrosis (257.3 \pm 21.6) (p=0.04). Alpha-fetoprotein level was statistically significantly higher in CHC patients than CHB and control groups.

Conclusions: This study showed increased levels of IL-1 β , IL-6 and TNF- α in CHB and CHC patients. TNF- α level further increased in patients with documented liver fibrosis.

Introduction

Hepatitis B (HBV) and hepatitis C (HCV) viruses are important health problems because they cause serious consequences such as chronic hepatitis, cirrhosis, fulminant hepatitis, and hepatocellular carcinoma (HCC) (1). Chronic liver disease occurs as a result of the relationship between a progressive wound healing process and inflammatory response (2). The mechanism of persistent and progressive HBV infection is not clear yet, and it is thought that host immune and genetic factors may play an important role (3).

Cytokines play a fundamental role in the immunopathogenesis of HBV infection and may affect the susceptibility to HBV infection and the natural course of the infection (4). HCV infection stimulates the production of inflammatory cytokines and chemokines, resulting in hepatic inflammation and chronic hepatitis (5). Many cytokines that affect the progression of liver disease and play an important role in the fibrotic process have been reported. Cytokines can reduce viral replication and control the host immune response. Accordingly, it can be said that the serum level of cytokines affects the outcome of the disease (6).

Interleukin (IL)-1 β is one of the powerful proinflammatory cytokines, a multifactorial inflammatory cytokine that has a central role in host defense and is an important immune response regulator (5). High IL-6 level may reflect more active hepatic necroinflammation. Therefore, it is a sensitive index for disease severity and progression (7).

Tumor necrosis factor (TNF)- α is a pro-inflammatory and antiviral cytokine secreted by macrophages and cytotoxic T lymphocytes in the liver, regulating immune reaction, cell growth and apoptosis. Therefore, TNF- α expression is considered to be an important molecular link between liver inflammation, steatosis, and fibrosis (8).

The aim of this study was to examine the changes in serum levels of IL-1 β , IL-6 and TNF- α cytokines in patients with chronic hepatitis B (CHB) and chronic hepatitis C (CHC), and the relationship between these cytokines with chronic inflammation, disease progression and fibrosis.

Methods

Patients and Controls

In this prospective clinical research study, a total of 90 CHB patients, 40 CHC patients, and a 50 controls without a history of hepatitis B surface antigen (HBsAg), anti-HCV and anti-human immunodeficiency virus (HIV) negative, acute and chronic hepatitis and any chronic disease, who came to the unit of infectious diseases between august 2016 and august 2018, were included. When the study started, CHB and CHC patients had not taken any medication.

Patients with a co-infection of hepatitis A and HCV, HIV patients who could not undergo liver biopsy, patients with positive autoimmune serology, and patients with HCC and cirrhosis were excluded from the study. The diagnosis of CHB and CHC was made according to the criteria of the European Association for the Study of the Liver (with laboratory and pathological evaluation) (9).

Data Collection

All of the CHB and CHC patients underwent liver biopsy. Liver biopsy specimens were scored using the Ishak histological scoring system (fibrosis was evaluated out of 6). CHB and CHC cases were divided into two groups as prominent fibrosis (stage 3-4) and mild fibrosis (stage 1-2) (since there were no stages 5 and 6).

As a part of routine patient analysis, the standard blood tests that were performed included HBsAg, HBV viral load (HBV DNA), HCV viral load (HCV RNA) serum alanine aminotransferase and aspartate aminotransferase, gamma glutamyl transferase, and alpha-fetoprotein (AFP) levels. Liver biopsy was performed as a part of routine clinical assessment (in HBV DNA >2000 IU/mL and HCV RNA positive cases).

Determination of Serum Levels of IL-1 β , IL-6, TNF- α

Approximately 5 cc of blood was drawn from the peripheral venous blood from the patients and separated into ethylenediamine tetraacetic acid-containing tubes. Within 40 minutes after collection, the obtained blood samples were centrifuged for 10 minutes at 3500 rpm. Serums were stored at -80 °C in a deep freezer. Serums were brought to room temperature and melted in weekdays. Serum IL-6, IL-1 β , and TNF- α levels were determined by the enzyme-linked immunosorbent assay method (R&D Systems, Minneapolis, MN, USA). Test results are expressed in ng/mL.

The study protocol was in accordance with the Helsinki Declaration of ethics and the study was approved by the Firat University Clinical Research Ethics Committee (Code: 14.12.2017/05). Patients' informed consent was obtained with the form.

Statistical Analysis

Data analysis procedures were carried out using Statistical Package for the Social Sciences 22.0 (Chicago, USA) package statistics software. The Kolmogorov-Smirnov and Shapiro-Wilk normality analyses were performed to determine the conformity of continuous variables to normal distribution. Student's t-tests were used in the analysis of continuous variables conforming to normal distribution. The "chi-square test" was used in the analysis of categorical data. Numerical data were expressed as mean \pm standard deviation, and categorical data as %. One-way ANOVA (Tukey, Bonferroni) test was used for multiple comparisons. P<0.05 value was considered significant in statistical comparisons.

Results

The final sample included 90 patients with CHB, 40 patients with CHC and 50 controls. Overall, 49 of CHB cases were female while 41 were male (age range: 42.6 \pm 11.8). There were 15 female and 25 male (age range 45.12 \pm 13.6) cases of CHC, while the control group consisted of 30 female and 20 male cases (age range: 39 \pm 15.04). There was no statistically significant difference between CHB, CHC and control groups in terms of age, gender and biochemical parameters (p>0.05). The demographic characteristics of CHB and CHC patients and the control group are shown in Table 1.

IL-1 β , TNF- α and IL-6 serum levels were higher in CHB and CHC cases than in the control group and this was statistically significant [IL-1 β (ng/mL): 133.7 \pm 37.0, 125.92 \pm 12.7, 85.7 \pm 9.8; TNF- α (ng/mL): 307.9 \pm 68.9, 286.0 \pm 43.2, 72.0 \pm 14.01; IL-6 (ng/mL): 50.6 \pm 10.1, 55.07 \pm 9.79, 8.85 \pm 7.07, respectively] (p=0.01). However, there was no significant difference between CHB and CHC cases in terms of the levels of these cytokines (p=0.22, p=0.20, p=0.10, respectively). Serum IL-1 β , TNF- α and IL-6 levels and p values of CHB, CHC cases and control group are shown in Table 2.

Table 1. Demographic characteristics of chronic hepatitis B, chronic hepatitis C cases and control group, average values of laboratory tests

	CHB (n=90)	CHC (n=40)	Control (n=50)	p value
Age	42.6±11.8	45.1±13.6	39±15.0	0.24
Gender (female/male)	49/41	15/25	30/20	0.27
AST (IU/mL)	28.4±18.0	25.0±11.7	21±7.76	0.23
ALT (IU/mL)	40.7±41.3	27.4±19.0	23.7±14.8	0.32
GGT (IU/mL)	22.8±19.9	30.3±24.9	21.9±13.1	0.32
AFP (µg/L)	2.19±1.09	4.03±2.94	2.15±1.03	0.04
Total bilirubin	0.53±0.33	0.63±0.35	0.43±0.25	0.13
PTZ	11.6±0.84	11.7±1.03	11.1±1.36	0.18
PLT (x10 ³ /µL)	274±95.8	301±10	231±23.6	0.12
HBV DNA (10 ³ IU/mL)	85189±372100			
HCV RNA (10 ³ IU/mL)		5602.7±2947.5		

Data were expressed as mean±standard deviation. AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, GGT: Gamma glutamyl transferase, AFP: Alpha fetoprotein, PTZ: Prothrombin time, PLT: Platelet, HBV: Hepatitis B virus, HCV: Hepatitis C virus

Table 2. Serum cytokine levels and p values of chronic hepatitis B, chronic hepatitis C cases and control group

	CHB (n=90)	CHC (n=40)	Control (n=50)	p1	p2
IL-1β (ng/mL)	133.7±37.0	125.92±12.7	85.7±9.8	0.01	0.22
TNF-α (ng/mL)	307.9±68.9	286.0±43.2	72.0±14.01	0.01	0.20
IL-6 (ng/mL)	50.6±10.1	55.07±9.79	8.85±7.07	0.01	0.10

Data were expressed as mean±standard deviation. p1: p values between CHB, CHC and control group; p2: CHB-CHC p value.
CHB: Chronic hepatitis B, CHC: Chronic hepatitis C, IL: Interleukin, TNF: Tumor necrosis factor

In CHC and CHB cases, there was no statistically significant difference in serum levels of IL-1β and IL-6 cytokines between patients with significant fibrosis and mild fibrosis (IL-1β: p=0.44, p=0.49; IL-6: p=0.50, p=0.38, respectively). However, TNF-α (ng/mL) level of patients with CHC with significant fibrosis (320.5±36.9) was significantly higher than that with mild fibrosis (257.3±21.6) (p=0.04). Serum TNF-α levels of CHB cases were higher in those with significant fibrosis than those with mild fibrosis. However, this was not statistically significant (p>0.05). IL-1β, TNF-α and IL-6 serum levels and p values according to the fibrosis scores are shown in Table 3.

AFP (µg/L) levels of patients with CHC (4.03±2.94) were statistically significantly higher than those of the patients with CHB (2.19±1.09) (p=0.01) and control groups (2.15±1.03 µg/L) (p=0.01).

Discussion

Despite advances in medicine and technology, morbidity and mortality rates of CHB and CHC are high, especially in developing countries (10). In recent studies, it has been reported that the increase in proinflammatory cytokines such as IL-1β, TNF-α,

and IL-8 may be effective in the development of tumors and chronic inflammatory diseases (11,12). In chronic viral hepatitis cases, increased cytokine levels cause inflammation in the liver (1). Therefore, measuring cytokine levels provides useful information about the activity and prognosis of the disease (13).

In a study conducted on patients with CHC, it was reported that the increase in IL-1β activity caused the development of fibrosis and inflammation to be more severe. In addition, it is stated that IL-1β plays a role in the pathogenesis of chronic hepatitis by reducing interferon-induced antiviral activity (1). It has been suggested that IL-1β mediates immune responses by inducing other proinflammatory genes. There are several studies documenting increased serum IL-1β levels in CHB cases (14). Watashi et al. (15) have shown that IL-1 can protect against HBV infection.

It has been suggested that the high production of IL-1β can help increase the production of other cytokines such as IL-2, IL-6 and TNF-α and trigger complex immunological processes to eradicate the virus (16). In this study, in accordance with the studies, IL-1β serum levels were statistically significantly higher in CHB and CHC than in the control group (p=0.01). This may indicate that IL-1β may be effective in the pathogenesis of CHB and CHC.

IL-6 is a multifunctional cytokine, important in inflammation, cell differentiation and tumor development (11,17). Levels of IL-6 have been shown to be significantly higher in CHB patients than in healthy humans and are expressed at significantly higher levels in those with severe liver disease (12,18). Again, a number of studies have shown that serum levels of IL-6 are increased in patients infected with HBV and are significantly higher in patients with severe acute infection than in patients with chronic active infection (2).

Table 3. Interleukin (IL)-1 β , tumor necrosis factor- α and IL-6 serum levels and p values according to the fibrosis scores

	CHB (n=90)			CHC (n=40)		
	Mild fibrosis (stage 1-2)	Significant fibrosis (stage 3-4)	p value	Mild fibrosis (stage 1-2)	Significant fibrosis (stage 3-4)	p value
IL-1 β	121.7 \pm 13.0	124.4 \pm 7.69	0.44	130.5 \pm 14.3	69.6 \pm 81.9	0.49
TNF- α	302.3 \pm 68.9	317.3 \pm 24.2	0.46	257.3 \pm 21.6	320.5 \pm 36.9	0.04
IL-6	54.0 \pm 12.9	51.2 \pm 4.69	0.50	57.7 \pm 6.65	48 \pm 5.65	0.38

Data were expressed as mean \pm standard deviation. CHB: Chronic hepatitis B, CHC: Chronic hepatitis C

It has been reported that IL-6 levels are high in autoimmune and chronic inflammatory diseases, and therefore IL-6 can be a good marker for disease progression associated with HBV. Various studies have shown that IL-6 can suppress HBV replication and inhibit HBV entry (6). Kuo et al. (19) demonstrated that IL-6 suppresses HBV proliferation in the HBV replication cell line. Hösel et al. (20) showed that IL-6 participates in the inhibition of HBV replication in hepatocytes, provides early control of the virus, and limits the adaptive immune response.

Many studies have shown that serum IL-6 levels increase in the progression of HBV disease. It has been reported that IL-6, the main immunomodulatory cytokine, plays an important role in CHB pathology and that increased levels of IL-6 are an index of increasing disease severity (21). In another study, it was stated that serum IL-6 levels were increased in HCV-infected patients compared to healthy controls (22). In this study, in accordance with the studies, IL-6 serum levels were higher in cases with CHB and CHC than in the control group and this was statistically significant ($p=0.01$).

Produced in the liver and many organs, TNF- α plays an important role in all types of viral hepatitis and participates in the regeneration of liver cells in CHC. Serum concentration increases significantly in chronic hepatitis (9). In many studies conducted with CHC patients, TNF- α concentration values were found to be significantly higher than in healthy individuals (23). Neuman et al. (24) found that serum TNF- α values were significantly higher in patients with CHB than in patients with CHB and in patients with CHB than in the control group.

Recent publications have observed high levels of TNF- α in the serum of HCV-infected patients. It has been reported that the increase in TNF- α levels may also cause an increase in other proinflammatory cytokines such as IL-6 in HCV infection (25). TNF- α levels increase in serum and liver tissues of patients with CHB and CHC infection (9). Some studies have found significant relationships between serum TNF- α levels and hepatic inflammation (4).

In this study, in accordance with the studies, serum levels of TNF- α were significantly higher in cases with CHB and CHC than in the control group ($p=0.01$). In addition, TNF- α was significantly higher in patients with CHC than those with significant fibrosis and those with mild fibrosis.

AFP is a marker used to predict HCC development not only in patients with cirrhosis but also in HCV-infected chronic hepatitis patients (26). In this study, the AFP levels of the patients with CHB were statistically significantly higher than in the patients with CHB ($p=0.01$) and the control group ($p=0.01$) (12).

The study has some limitations. There was no patient group with fibrosis 5 and 6 and HCC. The study had a small sample size, especially a few cases with KHC.

Conclusion

In conclusion, we found higher IL-1 β , IL-6 and TNF- α levels in patients with CHB and CHC compared to healthy individuals. The findings suggest that these cytokines may play a role in chronicity and hepatic inflammation and immunosuppression, and may also affect the progression of CHB and CHC. Besides, circulating TNF- α level can be used for early detection of fibrosis and determining prognosis in cases with CHC. Nevertheless, these findings need to be explored in larger studies.

Ethics

Ethics Committee Approval: The study protocol was in accordance with the Helsinki Declaration of ethics and the study was approved by the Firat University Clinical Research Ethics Committee with the decision dated 14.12.2017 and numbered 05.

Informed Consent: Patients' informed consent was obtained with the form.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: A.Ş., Design: A.Ş., N.Ö.A., Data Collection or Processing: A.Ş., Ö.A.S, N.Ö.A., Analysis or Interpretation: A.Ş., Ö.A.S, N.Ö.A, Literature Search: A.Ş., Writing: A.Ş.

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References

1. Borekci G, Karakas Celik S, Kandemir O, Aras N, Yalin S. Kronik hepatit B ve C hastalarında IL-1 beta, IL-1 reseptör antagonisti ve IL-8 gen polimorfizmlerinin araştırılması [Investigation of IL-1 beta, IL-1 receptor antagonist and

- IL-8 gene polymorphisms in patients with chronic hepatitis B and C]. *Mikrobiyol Bul.* 2014;48:271-282.
2. Batailler R, Brenner DA. Liver fibrosis. *J Clin Invest.* 2005;115:209-218.
 3. Chang L, Lan T, Wu L, Li C, Yuan Y, Liu Z. The association between three IL-6 polymorphisms and HBV-related liver diseases: a meta-analysis. *Int J Clin Exp Med.* 2015;8:17036-17045.
 4. Motawi T, Shaker OG, Hussein RM, Houssen M. Polymorphisms of α 1-antitrypsin and Interleukin-6 genes and the progression of hepatic cirrhosis in patients with a hepatitis C virus infection. *Balkan J Med Genet.* 2017;19:35-44.
 5. Abdel-Latif MS. Plasma Levels of Matrix Metalloproteinase (MMP)-2, MMP-9 and Tumor Necrosis Factor- α in Chronic Hepatitis C Virus Patients. *Open Microbiol J.* 2015;9:136-140.
 6. Heidari Z, Moudi B, Mahmoudzadeh Sagheb H, Moudi M. Association of TNF- α Gene Polymorphisms with Production of Protein and Susceptibility to Chronic Hepatitis B Infection in the South East Iranian Population. *Hepat Mon.* 2016;16:e41984.
 7. Xia C, Liu Y, Chen Z, Zheng M. Involvement of Interleukin 6 in Hepatitis B Viral Infection. *Cell Physiol Biochem.* 2015;37:677-686.
 8. Bader El Din NG, Farouk S, El-Shenawy R, et al. Tumor necrosis factor- α -G308A polymorphism is associated with liver pathological changes in hepatitis C virus patients. *World J Gastroenterol.* 2016;22:7767-7777.
 9. European Association for the Study of the Liver. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. *J Hepatol.* 2017;67:370-398.
 10. Bilgiç Y, Harputluoglu M, Yaprak B, et al. The Relationship Between Hepatic Activity Index and Serum Tumor Necrosis Factor Alpha Levels in Patients with Chronic Active Hepatitis B and Chronic Active Hepatitis C. *Medicine Sci.* 2015;4:2772-2781.
 11. Okamoto K, Ishida C, Ikebuchi Y, et al. The genotypes of IL-1 beta and MMP-3 are associated with the prognosis of HCV-related hepatocellular carcinoma. *Intern Med.* 2010;49:887-895.
 12. Zimmermann HW, Seidler S, Gassler N, et al. Interleukin-8 is activated in patients with chronic liver diseases and associated with hepatic macrophage accumulation in human liver fibrosis. *PLoS One.* 2011;6:e21381.
 13. Conde SR, Feitosa RN, Freitas FB, et al. Association of cytokine gene polymorphisms and serum concentrations with the outcome of chronic hepatitis B. *Cytokine.* 2013;61:940-944.
 14. Lei Q, Li T, Kong L, et al. HBV-Pol is crucial for HBV-mediated inhibition of inflammasome activation and IL-1 β production. *Liver Int.* 2019;39:2273-2284.
 15. Watashi K, Liang G, Iwamoto M, et al. Interleukin-1 and tumor necrosis factor- α trigger restriction of hepatitis B virus infection via a cytidine deaminase activation-induced cytidine deaminase (AID). *J Biol Chem.* 2013;288:31715-31727.
 16. Saxena R, Chawla YK, Verma I, Kaur J. Interleukin-1 polymorphism and expression in hepatitis B virus-mediated disease outcome in India. *J Interferon Cytokine Res.* 2013;33:80-89.
 17. Ciurtin C, Stoica V. Hepatitis virus C infection, adipokines and hepatic steato-fibrosis. *J Med Life.* 2008;1:49-54.
 18. Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. *Cold Spring Harb Perspect Biol.* 2014;6:a016295.
 19. Kuo TM, Hu CP, Chen YL, et al. HBV replication is significantly reduced by IL-6. *J Biomed Sci.* 2009;16:41.
 20. Hösel M, Quasdorff M, Wiegmann K, et al. Not interferon, but interleukin-6 controls early gene expression in hepatitis B virus infection. *Hepatology.* 2009;50:1773-1182.
 21. Saxena R, Chawla YK, Verma I, Kaur J. IL-6(-572/-597) polymorphism and expression in HBV disease chronicity in an Indian population. *Am J Hum Biol.* 2014;26:549-555.
 22. Sghaier I, Mouelhi L, Ghazoueni E, Brochot E, Almawi WY, Yacoubi-Loueslati B. Role of TLRs and IL-6 in the outcome of chronic hepatitis C treatment in Tunisian population. *Cytokine.* 2017;99:297-304.
 23. Tang S, Liu Z, Zhang Y, et al. Rather than Rs1800796 polymorphism, expression of interleukin-6 is associated with disease progression of chronic HBV infection in a Chinese Han population. *Dis Markers.* 2013;35:799-805.
 24. Neuman MG, Benhamou JP, Marcellin P, et al. Cytokine-chemokine and apoptotic signatures in patients with hepatitis C. *Transl Res.* 2007;149:126-136.
 25. Sevastianos VA, Voulgaris TA, Dourakis SP. Hepatitis C, systemic inflammation and oxidative stress: correlations with metabolic diseases. *Expert Rev Gastroenterol Hepatol.* 2020;14:27-37.
 26. Tateyama M, Yatsuhashi H, Taura N, et al. Alpha-fetoprotein above normal levels as a risk factor for the development of hepatocellular carcinoma in patients infected with hepatitis C virus. *J Gastroenterol.* 2011;46:92-100.