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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

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Abstract

In the present study, we investigated the relationship between serum TNF-α levels and hepatic activity in liver biopsies of chronic hepatitis B and C patients.The study was performed on 30 chronic hepatitis B patients, 25 chronic hepatitis C patients and 25 healthy controls. Serum samples of patients who underwent biopsy and healthy controls were collected. Control group was seronegative for hepatitis and had normal liver function tests. TNF- α levels measured by ELISA. Knodell's hepatic activity index was used in evaluation of liver biopsies. Serum TNF-α levels were determined higher in chronic hepatitis B and C patients than control group. HAI and TNF- α compared in chronic hepatitis B and C patients. Although we detected a relationship between HAI and TNF-α levels in chronic hepatitis B patients, no true correlation was shown in chronic hepatitis C patients. Cytokines have an important role in progression of chronic hepatitis B and C infections. There is a relationship between hepatic activity index and TNF-α level in chronic hepatitis B infection.But There is not relationship between hepatic activity index and chronic hepatitis C infection.only in two patients and both of them where in the middle of their reproductive age while as symptomatic ovarian cysts was a more common finding in unmarried females in 21-28 years of age group. This study concluded that laparoscopy has a diagnostic as well as a therapeutic implication in management of NSAP.

Keywords: Hepatic activity index, serum tumor necrosis factor alpha, chronic active hepatitis-b, chronic active hepatitis-c

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Introduction

In the world, the most common causes of chronic liver diseases are chronic hepatitis B and C. Hepatitis B virus is first defined in 1966, and currently more than 400 million individuals are believed to be chronically infected by this agent. Worldwide estimation of individuals infected by hepatitis C virus is approximately 190 million [1,2].

Despite all improvements in medicine and technology, both hepatitis B and C have high morbidity and mortality rates especially in underdeveloped and developing countries. Therefore, they are still important public health problems in those countries. Majority of infected individuals have progressive disease, and they develop late stage liver failure [3,4].

As it is the case in all chronic diseases, follow-up is mandatory in chronic liver diseases. The indispensable method of current clinical practice for disease follow up, and prediction of required intervention in time, is liver biopsy which provides significant information about liver histopathology, and stage of fibrosis. Disadvantages of liver biopsy may be named as it is an invasive procedure; it requires an expert pathologist; patient concerns and its possible complications. Therefore, non-invasive methods have aroused more interest in the follow-up of chronic liver diseases in today's medical practice, which employs minimal invasive approaches, radiology and biochemistry laboratory facilities more readily, and they have been subjects of many recent trials.

Although serum aspartate transaminase (AST) and alanine aminotransferase (ALT) values are the most commonly used investigations in estimating stage of chronic liver disease, studies have also been performed in many biochemical parameters [5,6].

The aim of the present study was to examine whether there was any correlations between tumor necrosis factor alpha (TNF-α) which is synthesized by monocyte/macrophages, mast cells, basophils, eosinophils, B and T cells, and inflammatory activity index in patients with chronic active hepatitis B and C; and to define whether there was any correlations between TNF-α and prognosis, disease activity, treatment response of patients with hepatitis.

Materials and Methods

This present study was performed on patients with chronic active hepatitis B and C infections who applied to the Gastroenterology Clinic and were diagnosed after investigations. The study was approved by the local ethics committee, and informed consents of all participants were provided before the study.

After clinical, laboratory, and histological examination of liver biopsy samples were performed in patients with chronic active hepatitis B and C infections, patients with alcoholic liver disease, autoimmune hepatitis, primary biliary cirrhosis, cholestasis, hepatitis B delta superinfection, and liver diseases of unknown etiology were excluded from the study. The study group was composed of 30 patients diagnosed with chronic active hepatitis B, and 25 patients diagnosed with chronic active hepatitis C infections. In the control group, 25 healthy individuals who were gender and age matched with hepatitis patients, and had negative viral panel, normal liver enzymes, and no alcohol and hepatotoxic drug use history, were included.

According to blood samples for hepatitis B infection, 30 patients with positive HBsAg antigen (HBsAg; Austria II, RIA kit, Abbott Laboratories, North Chicago, III, USA), and negative anti-HCV were enrolled in the study. According to chronic hepatitis C infection, 25 patients with negative HBsAg, and positive anti-HCV (second-generation enzyme-linked immunosorbent assay (ELISA); Abbot) were enrolled in the study. Viral nucleic acid study was performed one week before or after liver biopsy. Serum HBV DNA was evaluated by southern blot hybridization method, and HCV RNA was examined by Amplicor (Amplicor HCV test, Roche Diagnostic System INC. Asia, Singapore) test.

Liver Biopsy

Liver biopsy was performed percutaneously under abdominal ultrasonography guidance by using 18-G biopsy needle. Biopsy samples with the diameter greater than 2 cm were accepted as sufficient materials. After biopsy samples were fixed in 10% formaldehyde buffered solution for 24 hours, they were embedded within paraffin blocks, and sliced into pieces with 4-μm thickness. For evaluation of necroinflammatory activity, samples were stained by hematoxylin eosin and periodic acid-Schiff dyes. For evaluation of structural changes, samples were also stained by Masson trichrome and Sweet's reticulin dyes. Histological

changes were evaluated by a pathologist. Fibrosis and necroinflammatory activity were observed in biopsy samples, and they were organized according to Knodell's Histological Activity Index [7].

TNF-alpha Measurement

After 5 cc blood samples were obtained from patients with chronic active hepatitis and controls, they were centrifuged at 4000 for 5 minutes, and serums of patients were separated and stored at -80ºC. During serum study, samples were defrosted accordingly, and serum measurements obtained by TNF-α kit (Biosource International, CA, USA), were performed by using ELISA method.

Statistical Analysis

Statistical analysis was performed by using SPSS (SPSS 10.0 corp. Inc). One-way variance analysis (ANOVA) was performed for TNF-alpha values, and Duncan multiple comparison method was used to define differences between different group means. Kruskal-wallis test, a non-parametric test, was used to define differences mean values in HAI values between control, hepatitis B, and hepatitis C groups. Sperman's rho correlation test, one of non-parametric statistical methods, was used to test correlation between HAI and TNF-alpha values of patients.

Results

The mean values of laboratory tests are summarized in Table 1. The obtained data were tested by using one way variance test, and significant differences were determined in mean values of TNF-alpha values between control, chronic hepatitis B, and chronic hepatitis C groups ($p<0.001$). According to Duncan multiple comparison test, there were significant differences in TNF-alpha levels between control, chronic active hepatitis B and C groups. TNF-alpha levels were determined significantly higher in patients with chronic active hepatitis C than B; and in chronic active hepatitis B patients than the control group.

Table 1. Data of study groups.

Parameter		Chronic Active Hepatitis B	Chronic Active Hepatitis C	Control Group
N		30	25	25
Gender	**Male (%)**	18 (60%)	16 (64%)	18 (72%)
	Female (%)	12 (40%)	9 (36%)	7 (28%)
Mean age		35 (18-55)	27 (19-48)	28 (25-52)
ALT (IU/L)		104.2 ± 48.2	68.16 ± 39.21	19.76 ± 5.4
AST (IU/L)		75.5 ±43.5	45.8 ± 20.59	15.88 ± 6.51
HAI		8.03 ± 3.02	6.36 ± 2.46	0
TNF alpha		106.9 ± 98.9	165.34 ± 111.08	10.96 ± 4

According to Kruskal-Wallis test, there was no difference in HAI values between patients with hepatitis B and C; but HAI values in hepatitis B and C patients were statistically significantly higher than the control group ($p<0.0001$) (Table 2).

Table 2. Correlations between HAI values and subjects in control, chronic active hepatitis B, and chronic active hepatitis C groups.

Group	N	Mean ranking value	$\times^2$ Calculation value
Control	25	13.00 a	55.068
HAI (Hepatitis B)	30	57.22 b	55.068
HAI (Hepatitis C)	25	47.94 b	55.068

Spearman's rank correlation analysis was performed to determine whether there was a correlation between HAI and TNF-alpha levels in patients with chronic active hepatitis B and C. According to the test result, while there was a significant correlation between TNF-alpha levels and hepatic activity in patients with chronic active hepatitis B (r:0.423) (comment: it was shown that while one of them was increasing the other was decreasing) ($p<0.05$), the correlation was insignificant between TNF-alpha levels and hepatic activity among patients with chronic active hepatitis C (r:0.164) ($p>0.05$).

Discussion

Chronic active hepatitis B and C infections are still an important mortality and morbidity problem in the world. Complications of these infections may be improved if causes of chronic active hepatitis infections are defined, and it is clarified how the related complications are developed.

TNF-alpha is a procytokine which has a lethal role for cells produced in liver and other many organs, as well as causing programmed apoptosis of cells.

TNF-alpha is an important mediator of cellular immune response and inflammation. It has been shown that soluble TNF receptors (sTNFR) sTNF-R55 and sTNF-R75 levels were higher in infectious diseases (including HIV and hepatitis B virus) of TNF alpha were higher. Zylberberg et al. [8] investigated correlations between virological, biological, clinical, and histological characteristics of hepatitis C virus and TNF-alpha activity on 60 patients with chronic active hepatitis C infection. They reported that TNF-alpha levels in patients with chronic active hepatitis C were higher than the healthy controls; but there was no significant difference in TNF-alpha levels between patients with chronic active hepatitis B and C infections. In that study, serum TNF-alpha levels were determined significantly higher in patients with chronic active hepatitis B and C than the controls, but no significant difference was determined in TNF-alpha levels between patients with hepatitis B and C infections. Thus, the investigators showed that there was a correlation between TNF-alpha, especially sTNF-R75 levels, and disease activity. In the present study, it was also determined that TNF-alpha levels were higher in patients with chronic hepatitis C infection. This finding indicated that TNF-alpha might be one of immunological mechanisms related to disease activity in hepatitis-C infection related liver diseases.

Pathogenesis and chronicity mechanisms of chronic hepatitis B and C viruses are different. It is believed that chronicity of hepatitis B infection is related to immune tolerance, and liver damage which is evolved via immune mediated cytopathy, plays a role in the pathogenesis [9]. However, there is no immune tolerance in hepatitis C infection. It has been proved that humoral and cellular immunity play a role in its chronicity. In it spathogenesis, it is proposed that liver damage is caused both by direct viral effect and immune mediated cytopathy [10].

TNF-alpha levels are increased both in serums and liver tissues of patients with chronic hepatitis B and C infections [11]. In cell culture studies, signaling molecules formed by binding of TNF-alpha to TNFR1 are responsible for cytotoxicity of TNF-alpha [12]. TNFR2 increases cytotoxicity induced by TNFR1. TNFR2 alone does not cause cytotoxicity [13]. Tai et al. [14] reported that TNFR1 and hepatic activity index were correlated in 38 patients with chronic hepatitis B, whereas they were not correlated in 40 patients with chronic active hepatitis C. In the present study, TNF-alpha levels of chronic active hepatitis B and C patients were higher than the controls. While there was a significant correlation between HAI and serum TNF-alpha levels in patients with chronic active hepatitis B infection; there was no correlation in patients with hepatitis C infection. We believe that there is a strong correlation between hepatic activity index (HAI) and TNFR1 in chronic hepatitis B infection, whereas there is no such correlation in patients with chronic hepatitis C infection. Thus, we believe that TNF-TNFR1 signaling is different in liver cells infected by hepatitis B and C, so immunopathogenesis of the two viruses are different.

In the present study, we determined that TNF-alpha levels were higher in patients with chronic active hepatitis B infection than the control group. TNF-alpha gene polymorphism should be studied first in order to comment on progression of chronic active hepatitis B infection. TNF-alpha inhibits replication and expression of hepatitis B virus. Recombinant TNF-alpha increases damage of hepatitis B virus mRNA, and so inhibits HBV replication after the transplantation [15].

Zaigham Abbas et al. [16] reported no significant correlation between fibrosis and hepatic activity index and TNF-alpha and TNF-alpha gene polymorphism in hepatic biopsy samples of 40 patients with hepatitis C infection. In the present study, no significant correlation was determined between TNF-alpha levels and hepatic activity index in patients with chronic hepatitis C, and thus we believe that TNF-alpha does not have an important role in progression and activation of hepatitis C infection.

Zou et al. [17] reported in their study on 94 chronic liver patients and 31 healthy controls that serum TNF-alpha levels of chronic liver disease patients were higher; TNF-alpha levels were correlated with fibrosis; and TNF-alpha had a role in development of liver fibrosis. Kakumo S et al. reported that disease showed progression in chronic hepatitis patients who had high TNF

alfa1 (p55) and * TNF alfa 2 (p75) measured by ELISA, but there was no significant correlation between treatment response to interferon and levels of these receptors [18]. In the present study, as we determined higher levels of TNF-alpha in patients with chronic hepatitis B and C, we believed that TNF-alpha had an important role in hepatitis progression. However, we had no information about correlation between interferon treatment and TNF-alpha, because TNF-alpha levels were not measured after treatment in the patients.

TNF-alpha levels are increased in chronic hepatitis B infection. Additionally, TNF-alpha levels are intensively higher in chronic hepatitis B patients who receive interferon-gamma treatment, and transaminases are increased. Increases in TNF-alpha levels indicate hepatitis B virus elimination [19]. Akpolat et al. [20] showed that cytokines (including ATNF-alpha) played an important role in clinical picture and progression of chronic hepatitis in their study performed on 30 chronic hepatitis B infected patients. In the present study, TNF-alpha levels were determined high in patients with chronic hepatitis B. We believe that this increase may be significant in inhibiting viral replication, and elimination of hepatitis B virus. However, further studies are required to enlighten this issue.

After performance of partial hepatectomy (70% of the liver), TNF-alpha production is increased in the liver. In a TNF-alpha neutralization study via antibodies, it was observed that TNF-alpha I receptor was activated, and started liver regeneration after partial hepatectomy performed on TNF-alpha I receptor knock-out mice [21]. TNF-alpha, which acts with other inflammatory cells, causes hepatocyte death in early phase of many types of liver damages by triggering cytokine production, as well as initiating hepatocyte recovery including liver fibrosis. Therefore, proinflammatory cytokine TNF-alpha is a key factor in liver disease progression [22]. In the present study, TNF-alpha levels were higher in patients with chronic hepatitis B and C infections than the controls, but no correlation was detected between HAI and TNF-alpha levels in patients with chronic hepatitis C. Thus we believe that according to the state of immune system, high TNF-alpha levels are correlated with hepatocyte recovery in the early phase of liver disease in chronic hepatitis B infection, and also correlated to hepatocyte death and fibrosis in the late phase. However, we believe that this is not the same in patients with chronic hepatitis C infection.

In conclusion, serum TNF-alpha levels have been determined higher in patients with chronic hepatitis B and C. Pathogenetic mechanisms of both diseases are different, because serum TNF-alpha levels in chronic hepatitis B have correlated with HAI levels, whereas not in chronic hepatitis C infection. We believe that further studies are required investigating effects of TNF-alpha in liver cell damage and fibrosis. Currently, the requirement of predictive, non-invasive, and easily reproducible tests for liver damage is increasing. For this reason, retrospective studies investigating liver biopsy and biochemical findings may be guiding in this field.

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