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The Relationship Between Tumor Necrosis Factor Alfa Level and Hepatic Activity Index in Patients with Chronic Hepatitis C

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Hepatitis C virus (HCV) infection is a health problem all over the world. It is a major reason of cirrhosis and hepatocellular carcinoma. In this study, we aimed to examine the relationship between liver Hepatic Activity Index (HAI) and Tumor Necrosis Factor alpha (TNF alpha) levels in patients with chronic hepatitis C, and to determine whether there is a significant correlation. Thirty five chronic HCV patients, who were monitored by Infection Diseases and Clinic Microbiology Clinic, were taken into the study. Liver Biopsy samples were examined by the same pathologist and necroinflammatory activity was evaluated according to Knodell's classification. As the control group, 35 healthy volunteers without HCV infections were selected. TNF alpha was measured by ELISA method, for the serums acquired from bloods of patients and controls. TNF alpha and HAI levels was found to correlate in a positive, medium level, (r=0.379, p=0.02). In our study, we found a statistically significant relationship between HAI and TNF alpha levels in patients with chronic hepatitis C. We presume that serum TNF alpha level can be used to estimate the inflammation in the liver during HCV infection.

Key words: Chronic Hepatitis C, Tumor Necrosis Factor Alfa (TNF Alfa), Hepatic Activity Index (HAI).

The hepatitis C virus (HCV) is a member of the genus hepacivirus within the family Flaviviridae[1, 2]. HCV is mainly transmitted by transfusion of blood and blood products and percutaneous injury [3]. HCV infection is widespread across the world and represents a serious health problem. The global prevalence of HCV infection is 3%, and some 210 million people are infected with the virus [4,5]. Chronicity occurs in 50%-85% of people who experience acute infection [6]. Chronic hepatitis C (CHC) is the most important cause of cirrhosis and hepatocellular carcinoma [7].

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HCV is not a direct cytopathic virus, and liver damage is associated with immune-mediated mechanisms [7]. In chronic liver cell damage in patients with chronic hepatitis, TNF alpha is secreted by macrophages, inflammatory cells and damaged hepatocytes in the liver and plays a role in the apoptosis of hepatocytes. Various scoring systems are used to assess the degree of inflammatory damage in the liver. The hepatic activity index (HAI) developed by Knodell et al. is still widely used. This shows the degree of necroinflammatory activity [8]. The most significant undesirable outcome of CHC is hepatic fibrosis, and consequent cirrhosis and hepatocellular cancer [9].

The primary source of TNF alpha is macrophages and monocytes, other sources being

T and B lymphocytes, natural killer (NK) cells, neutrophils, astrocytes, kupffer cells, fibroblasts, mast cells, smooth muscle cells, epidermal cells and endothelial cells. TNF alpha secreted by these cells exhibits both autocrine and paracrine effects [10]. With its direct antiviral effect, immunomodulator activity, cytotoxic effect with apoptosis in virus-infected cells and multiple biological functions, TNF alpha is known to play an important role in inflammation and cellular immune response [11]. TNF alpha, mononuclear phagocytes and vascular endothelin stimulate IL-1 and IL-6, and hepatocytes stimulate synthesis of acute phase proteins. Excess TNF alpha secretion may lead to death through circulation failure and disseminated intravascular coagulation [12].

Cytokines' response to a virus can also result in liver damage. TNF alpha released from macrophages and hepatocytes that stimulate inflammatory response is important in the first response defense to hepatitis C. It exhibits a direct antiviral effect against HCV. At this point it increases lymphocyte proliferation and stimulates target specific CTL response [13].

Chronicity of the disease is closely associated with minimal or no T cell response to HCV. CD4+ and CD8+ T cell response to HCV proteins ends with resolution of the disease. In acute cases, significant CD4+ T cell proliferation occurs against several viral antibodies. Major histocompatibility complex (MHC) class II controls immune response to antigen-specific CD4+ cells. CD4+ cells are divided into two main groups: T helper (Th) 1 and 2 (Th1, Th2). Th1 cells support cytotoxic T lymphocyte (CTL) by releasing interleukin 2 (IL-2) and IFN gamma and provides defense against intracellular infections, while Th2 provides antibody response by releasing IL-4, IL-5, IL-10 and IL-13. CD4+ response increases production of B cells and CD8+ cells specific to virus-infected cells by providing both development of antiviral response and releasing cytokines during it[13,14].

The spread of HCV is basically carried out by T Suppressor (Ts) (CD8+) and T Helper (Th) (CD4+) cells. B lymphocytes present MHC class II molecules and viral peptides to CD4+ T lymphocytes. Cytokines released from these cells regulate B and CD8+ T cell activity. In the absence of CD4+ lymphocytes, immune response weakens and cytotoxic T lymphocyte (CTL) memory function cannot be maintained. CTL is responsible both for breakdown of infected cells and for TNF alpha and IFN gamma production [14,15]. Cytokine producing CD4+ T and CD8+ T cells play an important role in suppression of virus replication and in the formation of liver damage [16].

The purpose of this study was to determine whether or not TNF alpha is associated with prognosis and disease activity in patients with hepatitis by establishing a correlation between infection activity index and TNF alpha in patients with CHC. If a direct correlation is established between HAI and TNF alpha, then serum TNA alpha levels can be used in estimating inflammation in clinical practice.

MATERIALS AND METHODS

Thirty-five chronic HCV positive(+) patients under monitoring by the Infectious Diseases and Microbiology Clinic between 2009 and 2011 were included in the study following receipt of Atatürk University Medical Faculty Ethical Committee approval and informed consent from subjects (Group C: Chronic HCV Infection Group, n=35). Ultrasound (USG)-guided liver needle biopsy was performed on all patients in the radiology clinic. Biopsy specimens were sent to the Atatürk University Medical faculty Pathology Department laboratory under appropriate conditions. Biopsy materials were examined by the same pathologist. Necroinflammatory activity was assessed based on the Knodell classification. Thirty-five healthy volunteers, of similar age and gender to the study group, anti-HCV negative and with hepatic enzymes within normal limits were enrolled as the control group (Group K: Control Group, n=35).Cases aged under 18 or over 65, cases with auto immune chronic hepatitis, with liver disease of uncertain etiology or with HBV infection accompanying chronic HCV infection were excluded.

Virological Tests

Thirty-five HBs Ag (hepatitis B surface antibody) negative and anti-HCV (second-generation enzyme-linked immunosorbent assay (ELISA); Abbot) positive patients were enrolled

for CHC infection. Serum HCV RNA was assessed using the Amplicor (Amplicor HCV test, Roche Diagnostic System INC. Asia, Singapore) test.

TNF Alpha Measurement

Five cubic-centimeter venous blood specimens from patients diagnosed with CHC and from the control group were placed in biochemistry tubes. After 30 min, specimens were centrifuged for 5 min at 4000 g. Serum specimens were placed into Eppendorf tubes and immediately stored at -80 degrees. Sera were thawed according to protocol, and TNA alpha levels from serum were measured using ELISA with a TNF alpha kit (Invitrogen Human TNF Alpha).

Liver Biopsy and Histopathological Examinations

Patients were informed and consent was obtained before liver biopsy. USG-guided liver biopsy was performed in the radiology clinic, under percutaneous local anesthesia, using a 16-G or 18-G tru-cut automatic biopsy needle. Biopsy specimens larger than 2 cm were regarded as sufficient. Biopsy specimens were fixed for 24 h in 10% formaldehyde buffer solution and sent to the pathology laboratory. Specimens were stained with hematoxylin-eosin and periodic-acid Schiff stain for assessment of necroinflammatory activity. Specimens were stained with Masson's trichrome and Sweet's reticulin stain for assessment of fibrosis and structural changes. Histopathological changes were assessed by the same pathologist. Fibrosis and necroinflammatory activity in specimens were assessed on the basis of the Knodell classification.

Statistical Analysis

Statistical analyses were performed using Statistical Product and Service Solutions (SPSS) software version 18 (SPSS, Chicago, IL, USA). Student's t test, the chi square test, Spearman correlation analysis and the Mann-Whitney U test were used. Significance was set at p values less than 0.05.

RESULTS

Thirty-five cases diagnosed with CHC by the Atatürk University Medical Faculty Infectious Diseases and Microbiology Clinic and undergoing liver biopsy were included in the study. Thirty-

Table 1. Cases' Demographic Characteristics

		Grup C (n=35)	Grup K (n=35)	p value
Age	Mean±SD	49.2 ± 7.1	47.2 ± 7.1	0.24
	min-max median	27-60	32-60	
		50	47	
Gender	Male	19	17	0.63
	Female	16	18	

Group C: Chronic HCV Infection Group, Group K: Control Group. (p>0.05)

Table 2. TNF Alpha, ALT and AST Levels in the Chronic Hepatitis C and Control Group Cases

		GRUP C	GRUP K	p value
TNF-Alfa	Mean ± SD	10.7 ± 10.06	2.3 ± 1.03	0.00
	Min – Max	2.4 - 41.01	0.7 - 4.4	MU=55.0
	Median	7.3	2.2	
ALT	Mean ± SD	48.3 ±5.9	20.3 ± 0.78	0.00
	Min – Max	14-189	10-27	MU=206.5
	Median	36.0	22.0	
AST	Mean ± SD	47.2 ±5.3	17.7 ± 0.86	0.00
	Min – Max	15-145	10-30	MU=112.0
	median	38.0	18.0	

Group C: Chronic HCV Infection Group, Group K: Control Group.

five healthy individuals, similar in age and gender to the study group, with anti-HCV negativity and hepatic enzymes within normal limits were enrolled as the control group.

Cases' demographic characteristics are shown in Table 1. There was no significant difference between the groups in terms of age or sex (p>0.05).

TNF alpha, ALT and AST levels in the CHC and control groups are compared in Table 2.

TNF alpha, ALT and AST values were significantly higher in the cases with CHC compared to the control group (p>0.05).

Relations between TNF alpha levels and HVC RNA, ALT and AST levels in the cases with CHC are shown in Table 3.

No statistically significant correlation was determined between TNF alpha levels and HCV RNA, ALT and AST levels in patients with CHC (p>0.05).

TNF alpha levels and hepatic index levels in cases of CHC are shown in Table 4.

Table 3. Comparison of TNF Alpha levels and HVC RNA, ALT and AST levels in the Group C cases

		HCV RNA	ALT	AST
TNF alpha	r	0.08	0.15	0.05
	p	>0.05	>0.05	>0.05

(r= 0.00-0.24 weak correlation, r= 0.25-0.49 moderate correlation, r= 0.50-0.74 powerful correlation, r= 0.75-1.00 very powerful)

Table 4. Correlation between TNF Alpha and HAI Levels in Cases of Chronic Hepatitis C

		HAI	
	N	r	p value
TNF alpha	35	0,379	0,02

TNF-alpha: TumorNecrosisFactor Alfa, HAI: Hepatic Activity Index

Table 5. Various studies involving TNF alpha and HAI in chronic hepatitis C

Researcher	Patients	TNF alpha (patient-control) p value	TNF alpha-HAI p value	TNFR-HAI p value
Nick E. (71)	41 HCV + 44 control Grup	p=0.000		
Zylberg (72)	60 HCV + 60 control Grup	p =0.0001		0.01
Kallinowski (78)	105 HCV + 48 control Grup	p <0.0001	p <0.001	p <0.001
Crespo (76)	135 HCV + 75 control Grup	p =0.01	p >0.05	
Kasprzak (81)	22 HCV + 6 control Grup	p <0.02	p =0.04	
Akçam (84)	25 HCV + 30 control Grup	p =0.017	p=0.001	
Karabulut (77)	30 HCV + 10 control Grup	p <0.0001		
Neuman MG (83)	778 HCV +		p <0.001	
Kakumo S. (73)	71 HCV + 11 control Grup			p <0.0001
This study	35 HCV + 35 control Grup	p =0.00	p =0.02	

TNF alpha: Tumor Necrosis Factor Alfa,HAI:Hepatic Activity Index, TNFR: tumor necrosis factor receptor

DISCUSSION

Liver damage in viral hepatitis occurs as a result of immune response of cells infected with the virus. After infecting hepatocytes, the hepatitis virus causes an immune response specific to the viral proteins. Cytokines protect directly in viral infections such as HCV by preventing viral replication and indirectly by increasing the body's cellular immune response[17].

TNF alpha is an important cytokine of cellular immune response and inflammation. TNF alpha levels increase in serums and in hepatic tissues in CHC infections. TNF alpha has been shown to play a significant role in the development of fibrosis in the liver [18].TNF alpha levels in serum reflect the degree of inflammation in cases of chronic HCV [14].

Spanakiset al.[19] enrolled 41 patients with hepatitis C and receiving dialysis and 44 healthy individuals as a control group in order to determine the mechanisms involved in liver damage and compared study group hepatic biochemical values, levels of TNF alpha and other cytokines with those of the control group. TNF alpha levels were determined to be higher than those of the control group.

Zylberg et al.[20] investigated TNA alpha activity, TNF receptor (TNFR) levels and the virological, biological, clinical characteristics of HCV in 60 patients with chronic hepatitis C, 34 patients infected with Hepatitis B virüs and a 60-member healthy control group. The results revealed higher serum TNF and TNFR levels in patients with chronic hepatitis C infection compared to the control group, and a correlation between TNFR levels and HAI levels.

Kakumo S et al.[21] measured TNF alpha1 (p55) and TNF alpha2 (p75) levels using ELISA in patients with hepatitis C with normal ALT levels, patients with CHC and patients with hepatitis C-related cirrhosis of the liver and hepatocellular Ca. They reported that the disease exhibited progression in patients with high receptor levels but that there was no significant correlation between receptor levels and response to interferon therapy [21].

Huang et al.[22] investigated cytokine levels in 20 patients with acute hepatitis C, 43 with CHC, 40 with cirrhosis of the liver, 36 with hepatocellular Ca and anti-HCV (+) and 30 healthy subjects and determined higher TNF alpha in cirrhosis and hepatocellular canser. A correlation was observed between TNF alpha levels and prothrombin time, but none with AST and ALT levels. They concluded that TNF alpha levels indicate hepatic dysfunction better than other inflammation parameters.

Zou B. et al.[23] compared 94 patients with CHC, cirrhosis of the liver or primary hepatocarcinoma with a 31-member healthy control group and reported higher serum TNF alpha levels in the three patient groups than in the control group.

Crespo et al.[24] investigated TNF alpha, fibrosis and plasma leptin values in a study of 135 patients with CHC infection and 75 healthy controls. TNF alpha levels were higher than those in the control group. TNF alpha levels were higher in patients with advanced fibrosis (stage IV) and patients with chronic hepatitis C than in patients with mild fibrosis (stages I and II), but no correlation was determined between TNF alpha and HAI. TNF alpha, an inflammation parameter, was also elevated in our study. However, we did determine a correlation with HAI. Hepatocytes increase cytokine production capacities as a response in liver injury, including viral injury. This suggests a parallel between immune response and increased damage and cytokine level elevation. We drew no conclusion in this study since we did not examine the fibrosis relationship.

Karabulut et al.[25] compared various cytokine levels in 10 patients diagnosed with acute hepatitis C, 10 with CHC and 10 patients with CHC who had completed 6 months of interferon therapy with those of a control group of 10 healthy individuals and determined higher TNF alpha levels in all groups compared to the control group. TNF alpha levels also differed between the groups, the highest level being determined in the patients with acute hepatitis C. These differing results suggested that the groups' immune responses and stages of the disease might have been involved.

Kallinowski et al. [26]investigated TNF alpha, TNFR and HAI values in 105 patients with chronic HCV and a control group of 48 healthy subjects. They determined that TNF alpha and TRNF levels in patients with chronic HCV increased in correlation with HAI and interpreted

this as a reflection of histological activity.Similarly in our study, TNF alpha levels in our 35 patients with CHC were significantly higher than those of the healthy control group. Elevation in TNF alpha levels in our and other studies appears as a defense mechanism, and a direct antiviral effect and immune response occur together with an increase in TNF alpha levels the virus infected hepatocyte region. As a result of these effects, virus clearance and/or damage may occur together in the liver.

The hepatitis C core protein is multifunctional and part of the viral nucleocapsid. The hepatitis C virus core protein primarily enters into reaction with TNFR. After combining with TNFR, the hepatitis C core protein undergoes apoptosis through signal proteins, and cell death occurs. This cell death and inflammation causes liver damage, and the HAI that can be evaluated following liver biopsy is frequently employed as a scale for classifying liver damage in patients with chronic hepatitis [27].

Hepatic pathology developing in HCV infection is generally compatible with pathology in forms of viral hepatitis, and is therefore not pathognomonic. The 15-20 year process required for cirrhosis to develop under normal conditions is also needed for the removal of the extracellular matrix [28,29].

Kasprzak et al.[30] investigated 21 patients with chronic hepatitis C and a 6-member control group and determined higher TNF alpha levels than in the control group, while TNF alpha activity at tissue level was correlated with HAI.

Sobchaket al.[31] investigated cytokine levels and the relation with HAI in 22 patients with hepatitis C, and assessed HAI using the Knodell scale. Elevated TNF alpha levels were shown in the CHC group, and TNF alpha levels were correlated with intralobulardegeneration, hepatic necrosis and inflammatory infiltration in the portal area. No correlation was determined between HAI and transaminases.

Neuman MG et al.[32] investigated 778 patients and reported that cytokines played a critical role in immunity and inflammation in patients with CHC. They examined 59 patients with low fibrosis and a low Knodell HAI value, 372 with mild fibrosis and a mildly elevated Knodell HAI value, 270 with moderate fibrosis and a moderate Knodell HAI value and 77 with high fibrosis and a high Knodell HAI value. A positive correlation was observed between level of inflammation and TNF alpha level in non-cirrhotic patients.

Akçam et al.[33] compared cytokine levels, HAI and fibrosis levels in 25 patients with hepatitis C, 25 with hepatitis B and 30 healthy controls. A statistically significant correlation was observed between TNA alpha and HAI and fibrosis values in patients with both CHC and CHB ($p<0.05$). That study reported that cytokine investigation in both groups can be a marker of severity of disease and clinical progression.

Pasha et al.[34] examined the association between response to treatment and polymorphism in TNF alpha structure in patients with hepatitis C. From a total of 440 patients with hepatitis C, 220 with virological response with combination therapy (interferon+ribavirin) and 220 with no response were selected. Subjects with TNF alpha 308 polymorphism were observed to be resistant to combined antiviral therapy. It has been reported that the cytokine structure changes in subjects with TNF alpha gene polymorphism, that these subjects may have a predisposition to HCV infection and that their response to treatment is low. TNF alpha inhibitor treatment in patients with HCV seems to be safe [35].Various studies cited involving TNF alpha and HAI in CHC are summarized in Table 5.

TNF alpha levels in patients with CHC were higher than those of the group, and there was a correlation between serum TNF alpha levels and HAI assessed after liver biopsy in patients with CHC infection.

There are a number of limitations to our study. One is the absence of serotype determination. Other limitations include the low number of patients and the fact that liver biopsy is invasive and involves difficulties of its own. The Knodell score was employed in pathology since the Ishak scoring system was not in use at the time of the study. TNF alpha levels could also have been compared with responses to treatment. Despite these limitations, however, this prospective study will contribute new information to our understanding of fibrosis.

In conclusion, serum TNF alpha levels rise in patients with CHC, that rise is correlated with HAI, and TNF alpha levels can therefore reveal the level of liver damage in patients with CHC in a

non-invasive manner. We think that TNF alpha levels can provide useful information concerning necroinflammation status in the liver. TNF alpha levels can be investigated as an alternative when liver biopsy cannot be performed or if the patient refuses biopsy. New therapeutic paths with antagonism of TNF alpha levels can be considered in future studies.

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