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Serum profile of T helper 1 and T helper 2 cytokines in patients with chronic hepatitis C virus infection

Kronik hepatit C infeksiyonlarında yardımcı T hücre tip 1 ve tip 2 sitokinlerinin serum düzeyleri

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Background!aims: *T cell immunoregulatory cytokines may play a crucial role in the host response to hepatitis C virus infection. While T-helper type 1 cytokines are required for host antiviral immune responses, T-helper type 2 cytokines can inhibit the development of these effector mechanisms. The aim of the present study was to determine T-helper type 1 and T-helper type 2 cytokine levels in chronic hepatitis C infection.* ***Methods:*** *Serum levels of T-helper type 1 cytokine, interferon-gamma (IFN-y), and T-helper type 2 cytokines, IL-4 and IL-10 were measured in 30 patients with hepatitis C infection and 25 healthy controls using ELISA. Serum levels of alanine transaminase were also assessed in both patients and controls. Histologic activity score was evaluated in the chronic hepatic C infected patients.* ***Results:*** *Serum levels of IFN-Y were 59. 03±46. 24 pg/mL, IL-4 were 213. 59±135. 67 pg/mL and IL-10 were 106. 73+60. 85 pgImL in hepatitis C infected patients whereas in healthy controls they were found to be 61. 84±54. 87 pglmL, 67. 39±59. 74 pg/mL and 60. 14+50. 73 pg/mL respectively. Serum levels of IL-4, IL-10 but not IFN-jwere found to be significantly increased in chronic HCVpatients compared to those of control subjects.* ***Conclusions:*** *Our findings may suggest the involvement of Th2 cytokines in the pathogenesis of chronic hepatitis C virus liver disease.*

Key words: Chronic hepatitis C, cytokines, IFN-y, IL-4 and IL-10.

Amaç: *T hücre regülatuvar sitokinleri, vücudun hepatit C virüs infeksiyonlarına karşı geliştirdiği yanıtta önemli rol oynayabilirler. Yardımcı T hücre tip l sitokinleri, vücudun antiviral immün yanıtı için gerekli iken yardımcı T hücre tip 2 sitokinleri, bu etken mekanizmaların gelişimini inhibe edebilirler. Bu çalışmanın amacı, kronik HCV infeksiyonlarında yardımcı T hücre tip l ve 2 sitokinlerinin düzeylerini belirlemektir.* ***Yöntem:*** *Yardımcı T hücre tip l sitokinlerinden interferon (IFN)-gamma ve yardımcı T hücre tip 2 sitokinlerinden IL-4 ve IL-10 düzeylerine kronik hepatit C virüs infeksiyonu olan 30 hastada ve 25 sağlıklı kontrolde ELISA yöntemi ile bakıldı. Hasta ve kontrollerde, serum alanın transaminaz (ALT) düzeyleri de ölçüldü. Hasta grubunda, histolojik aktivite skoru değerlendirildi.* ***Bulgular:*** *Hepatic C virüs ile inpekte hasta grubunda serum IFN-gamma düzeyleri 59.03±46.24 pglmL, IL-4 düzeyleri 213.59±135.67pglmL, IL-10 düzeyleri 106.73±60.85 pglmL olarak bulunurken aynı sitokinlerin düzeyi sağlıklı kontrol grubunda sırası ile 61.84±54.87pg/mL, 67.39+59.74 pglmL, 60.14+50.73 pglmL, olarak saptandı. Serum IL-4 ve IL-10 düzeyleri sağlıklı kontrol grubuna göre anlamlı olarak yüksek bulunurken, her iki grubun IFN-gamma düzeyleri arasında anlamlı fark saptanmadı. Sonuç: Bulgularımız, kronik hepatic C virüs infeksiyonunun patogenezinde yardımcı T hücre tip 2 sitokinlerinin yer aldığını düşündürtmektedir.*

Anahtar kelimeler: Kronik hepatit C, sitokin, IFN-y, IL-4 ve IL-10.

INTRODUCTION

Hepatitis C virus (HCV) is one of the major causes of chronic hepatitis, cirrhosis and hepatocellular carcinoma, although the exact mechanism of hepatocellular damage in chronic HCV infection remains unclear (1). Although it has not been demonstrated in cell cultures, HCV could be directly cytopathic for infected hepatocytes (2). Recent experimental studies have supported the role of immune response mechanisms in liver injury of HCV infections (3). Cytokine producing CD4+ and CD8+ T cells may play a role in the suppression of viral replication and may contribute to the development of hepatocellular damage (4,5). T helper (Th) cells and T cell derived cytokines may be important in the host immune response. Activated T lymphocytes may be divided into two functional subsets, Thl and Th2 cells, on the basis of the immunoregulatory cytokines that they produce (6). Thl cytokines, including IL-2, interferon-gamma (IFN-y) and tumor necrosis factor beta (TNF-J3), promote a cell-mediated immune response whereas Th2 cytokines which produce

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IL-4, IL-5, IL-10 and IL-13 are involved in antibody-mediated immunity. It has been suggested that the pathogenesis and persistence of infections involve a Thl to Th2 cytokine switch (7). The Thl and Th2 responses have been to be cross inhibitory (8,9). It is particularly important that the Th2 cytokines, IL-4 and IL-10, can downregulate cell mediated immune effector mechanisms, which are important for host defense against intracellular parasitic and viral infections (10).

Recent studies have demonstrated conflicting results on the levels of Thl and Th2 cytokines in HCV infections (7,11-17). While some reports have demonstrated elevated levels of IL-2 and IFN-y (11,12), and IL-4 and IL-10 (7,13,14), others reported no increase in the levels of Thl (15-17) and Th2 cytokines (11,17).

The aim of this study was to investigate the serum levels of Thl and Th2 cytokines, their roles in the pathogenesis of chronic HCV infection and to analyze how these cytokines related to histologic findings and laboratory parameters.

MATERIALS AND METHODS

Thirty patients (17 male, 13 female; mean age: 37.48±11.35 years) with chronic HCV infection and 25 healthy subjects (13 male, 12 female; mean age: 34.24±10.44 years) were included in this study. The diagnosis of the patients with chronic HCV infection was established on the basis of clinical, laboratory, ultrasonographic and histopathologic findings. Patients with cirrhosis were excluded. Histological activity index (periportal, portal and lobular inflammation) was assessed according to Knodell et al. Patients with scores of (4-6) were graded as mild, (7-9) as moderate and (10-13) as severe. The healthy subjects had negative hepatitis serology, normal liver function tests and normal ultrasonographic findings. Hepatitis serology was determined by microparticule enzyme immune assay (Abbott, Chicago, IL). Hepatitis C Virus RNA was detected with iCycleriQ™ Multi-Color Real Time PCR Detection System using BIO-RAD (#Catalog: 170-8744). Serum IFN-y, IL-4, and IL-10 levels were measured by ELISA using commercially available kits according to the manufacturer's instructions (Genzyme Corporation, Cambridge, MA, USA). The kits were able to detect concentrations of as low as 3 pg/mL of IFN-y, 6 pg/mL of IL-4, and 5 pg/mL of IL-10. SPSS for windows was used for statistical calculations (9.0). Mean and standard deviation (SD) were calculated for all variables. The Mann-Whitney U test was used to compare IFN-y, IL-4, IL-10, and ALT levels between the cases and the controls. Discrete variables were evaluated with Chi-square test. Odds ratios (OR) and 95% confidence intervals (CI) were calculated for IFN-y, IL-4 and IL-10. Correlations between variables were assessed with Spearman's correlation test. A value of $P<0.05$ was accepted as significant. The cut off value for each cytokine was calculated by adding 2 standard deviation (2SD) to the mean of that cytokine value of the healthy controls.

RESULTS

All patients had positive HCV RNA with PCR. Histologic activity scores were mild in five patients (16.7%) moderate in 12 patients (40%) and severe in 13 patients (43.3%). Serum levels of IFN-y, IL-4, IL-10, and ALT in patients with chronic HCV infection and healthy controls are presented in Table 1 and are illustrated graphically in Figure 1. There were no statistical differences among the serum levels of IFN-y between patients with chronic HCV infection and healthy

Table 1. Serum Thl and Th2 cytokines and ALT levels in patients with chronic hepatitis C infection and in healthy controls.

	IFN-gamma (pg/L) Mean±SD (Range)	IL-4 (pg/L) Mean±SD (Range)	IL-10 (pg/L) Mean±SD (Range)	ALT (U/L) Mean±SD (Range)
Chronic Hepatitis C	59.03±46.24 (182)	213.59±135.67 (502.72)	106.73±60.85 (222.15)	120.13±67.15 (287)
Healthy Controls	61.84±54.87 (241)	67.39±59.74 (222.48)	60.14±50.73 (157.42)	22.92±7.25 (28)
P value	0.973	0.000	0.002	0.000

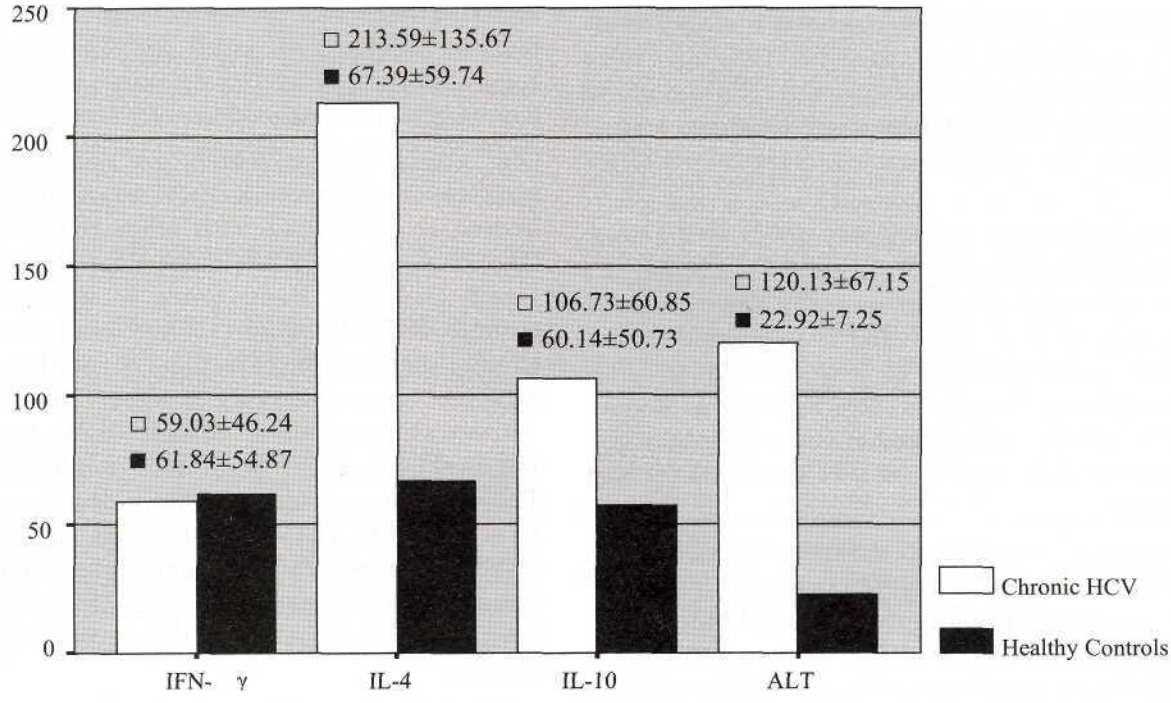

Figure 1. Illustration of serum Thl and Th2 cytokines and ALT levels in patients with chronic hepatitis C infection and in healthy controls.

controls (p>0.05), but serum IL-4 and IL-10 levels of the patients with chronic HCV infection were significantly higher than those of healthy controls (p<0.001 and p<0.002, respectively). Additionally, serum ALT levels of the patients with chronic HCV infection were found to be elevated when compared to those of healthy controls (p<0.001).

The cut off values for IFN- y, IL-4 and IL-10 were 171.58 pg/L, 186.87 pg/L, and 180.42 pg/L, respectively. Two (6.7%) out of 30 patients with chronic HCV infection and two (8%) out of 25 healthy controls had elevated IFN-y levels (p>0.05). While 13 patients (43.3%) and two healthy controls (8%) had elevated serum levels of IL-4 (p=0.003), three of the patients (10%) and none of the healthy controls had increased IL-10 levels in their sera (p>0.05). IFN-y, IL-4 and IL-10 positivities for HCV patient and healthy control groups, and their odds ratios and 95% CI are shown in Table 2.

There were significant correlations between IL-4 and IL-10 levels (r=0.391, p=0.032), between IFN-y and ALT levels (r=0.380, p=0.033), and between IL-10 and ALT levels (r=-0.520, p=0.003) in patients with chronic HCV infection. The serum levels of IFN-y, IL-4 and IL-10 were not correlated with the histologic activity score (p>0.05, for all comparisons).

Table 2. Thl and Th2 cytokines positivities in HCV patient and healthy control groups, and their odds ratios and 95% CI.

	HCV Patients n=30 p/n	Healthy Controls n=25 p/n	OR (95% CI), p value
IFN-gamma	2/28	2/23	0.8 (0.07-9.05) p=0.6
IL-4	13/17	2/23	8.8 (1.54-65.28) p=0.003
IL-10	3/27	0/25	3.22 (0.27-85.70) p=0.3

p / n : positive / negative

DISCUSSION

Cytokines are mediators which are mainly secreted by lymphocytes and monocytes. They have roles in the communication between the cells and in the regulation of immune response. They have a basic structure but exhibit complex biological activities (18).

Various mechanisms may play a role in the host-virus interactions and virus may escape from the host defensive mechanisms (19). One of these mechanisms is to inhibit the Thl cytokines at the mRNA synthesis level as with viral IL-10 since virus genome encodes a 70% homolog of human IL-10 (20). The other mechanism is to inhibit the transcriptional activity of IFN synthesis on host cells (21).

Our results revealed no difference in the serum levels of IFN-y between patients with chronic HCV infection and normal controls. On the other hand, Th2 cytokines, IL-4 and IL-10, were found to be higher in patients with chronic HCV infection than in healthy controls. None of the cytokines was correlated with the histological activity score.

Previous studies demonstrated various serum Thl/Th2 cytokine profiles in chronic HCV infection (11,12,15,16,22-24). It has been suggested that the differentiation of activated T cells toward Thl cells results in an improvement of hepatitis (24). Although serum levels of Thl cytokines, including IFN-y and IL-2 have been reported to be elevated in HCV infections (12), some others have shown low levels of IFN-y in patients with HCV infection (25). Osna et al measured serum IFN-gamma, IL-4, IL-10 and IL-12 levels and they found lower IFN-y and higher IL-10 levels in chronic HCV patients than in healthy controls. They also reported an increase in the serum levels of IFN-y when IL-10 production had been inhibited and suggested that IL-10 served as a downregulative factor for IFN-y in chronic HCV infection (15). Recently, Napoli et al. found that IFN-y and IL-2 mRNA were increased in the livers of patients with chronic hepatitis C, suggesting that these cytokines are locally produced by hepatic CD4+ cells. Moreover, the mRNA levels of IFN-y and IL-2 had been correlated with the extent of hepatic fibrosis and portal inflammation. They suggested the role of Thl cytokines in mediating hepatocellular damage (26).

Our study also revealed an enhanced Th2 response during chronic HCV infection and this finding is concordant with other recent studies (12,23). Reiser et al. demonstrated the elevated serum IL-4 and IL-10 levels in patients with chronic HCV infection (23). Cacciarelli et al showed that the concentrations of circulating Th2 cytokines were markedly greater than those of Thl cytokines during chronic HCV infection. Furthermore, IFN-y treatment diminished the Th2 cytokine response parallel to the decrease in viral load (12). The elevated serum Th2 cytokine levels may represent a systemic response or may be a result of increased local production within the liver which is secreted to the peripheral bloodstream.

On the basis of our data and previous reports, both Thl and Th2 cytokines have been involved in the pathogenesis of HCV infection. In fact, cell mediated immune response plays a predominant role in control of persistent viral infections and Thl cells are important for the local immune response of the host. Th2 cytokines regulate the antibody secretion by B cells and have suppressor functions. Increased levels of Th2 cytokines, IL-4 and IL-10, may be responsible for the decrease in IFN-y production in the present study and may be the cause of the chronicity of HCV infection. The outcome of the HCV infection is related to the replication rate of the virus and with the interactions between the virus and the host immune system (27).

In conclusion, our findings and those reported in the literature indicate that Thl and Th2 cytokines are somehow involved in the pathogenesis of chronic HCV infection. Further investigations are required to determine the significance of these cytokines in the development and disease progression of chronic HCV infection.

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