Arrival Date: 04.10.2021 | Published Date: 25.01.2022 | 2022, Vol: 7, Issue: 16 | pp: 23-29 | Doi Number: http://dx.doi.org/10.46648/gnj.299

Naiv Kronik Hepatit C Hastalarinda Serum Tümör Nekroz Faktör –Alfa, Transforming Growth Faktör –Beta, İnterlökin -10 Ve Alanine Aminotransferase Seviyeleri İle Tedavi Arasindaki İlişkinin Değerlendirilmesi

Evaluation Of The Connection Between Serum Tumor Necrosis Factor-Alpha, Transforming Growth Factor-Beta, Interleukin-10 And Alanine Aminotransferase Levels And Treatment In Naive Chronic Hepatitis C Patients

Barış Balasar[1], İbrahim Erayman[2]

ÖZET

Çalışmaya daha önce tedavi almamış (naiv) KHC'li hastalar dâhil edildi. Hastaların tamamı genotip 1b ve tamamı pegile interferon ve ribavirin kombine tedavisi başlanan hastalar idi. Hasta grubuna 43, kontrol grubuna 43 kişi dahil edildi. Hem hasta hemde kontrol grubundaki hastaların 23'ü erkek, 20'si kadındı. Hasta grubunun yaş ortalaması 49,54±13,02, kontrol grubunu yaş ortalaması 47,98±14,1 idi. Hasta ve kontrol gruplarından serum alanine aminotransferase (ALT), TNF-alfa, TGF- beta, IL–10 değerleri çalışıldı. TNF-alfa, TGF- beta ve IL–10 değelerini ölçmek için 10 cc venöz kan, parafin içeren biyokimya tüplerine alındı. Kanlar 5 dakika boyunca 5000 devirde santrifüj edildi. Bu işlemler 1 saat içerisinde gerçekleştirildi. Santrifüj işleminden sonra eppendorf tüpüne konulan serumlar çalışma gününe kadar -80 derecede derin dondurucuda saklandı. Serum sitokin düzeyleri kit prosedürleri uyarınca ELISA yöntemiyle ölçüldü. Human TNF-alfa (DIAsource TNF-alfa - ELISA Kiti), Human IL–10 (DIAsource IL–10-ELISA Kiti), Human TGF-beta ELISA- Multispecies TGF-beta kiti)' nın düzeyleri hasta grubunda tedavinin 0. 12. Ve 48. haftasındaki serum örneklerinden ve kontrol grubunda ise tek bir serum örneğinden çalışıldı. Serum örneklerinde saptanan değerler pg/ml olarak kaydedildi. Hasta ve kontrol grubunda serum ALT değerleri rutin olarak hastanemiz biyokimya laboratuvarında çalışıldı. Vaka grubuna kombine tedavi başlandı ve hastalar tedaviye yanıt açısından 12. ve 48. haftalarda değerlendirildi. Tedaviye yanıt olan ve olmayan olarak 2 gruba ayrıldı. Bu iki grubun TNF-alfa, TGF-beta ve IL–10 serum sitokin değerleri 0-12 hafta, 12-48 hafta ve 0-48. haftalarda kendi içlerinde ikili karşılaştırmalar yapıldı. Son olarak 0. 12. 48. haftalardaki sitokin değerleri ile ALT karşılaştırıldı. İstatistiksel analiz için Spearman'ın korelasyon testi kullanılmıştır. Yorumlamalarda anlamlılık sınırı $p<0.05$ alındı. İstatistiksel çözümlemelerde SPSS (sürüm: 17,0) paket programı kullanıldı. Tedaviye yanıt alınan hastalarda serum TNF-alfa, TGF-beta ve IL–10 serum sitokin değerlerinde istatistiksel fark bulunmuştur.

Anahtar Kelimeler: Biyolojik Belirteçler; Karaciğer sirozu; Kronik hepatit c; sitokinler

ABSTRACT

Patients with naive CHC were included in the study. All of the patients were genotype 1b and all of them were treated with pegylated interferon and ribavirin combined. 43 people were included in the patient group and 43 people in the control group. Of the patients in both the patient and control groups, 23 were male and 20 were female. The mean age of the patient group was 49.54±13.02, and the mean age of the control group was 47.98±14.1. Serum alanine aminotransferase (ALT), TNF-alpha, TGF-beta, IL-10 values were studied in the patient and control groups. To measure TNF-alpha, TGF-beta and IL-10 values, 10 cc of venous blood was taken into biochemistry tubes containing paraffin. Blood was centrifuged at 5000 rpm for 5 minutes. These procedures were carried out within 1 hour. After centrifugation, the serums placed in the Eppendorf tube were stored in a deep freezer at -80 degrees until the study day. Serum cytokine levels were measured by ELISA method according to kit procedures. Levels of Human TNF-alpha (DIAsource TNF-alpha - ELISA Kit), Human IL-10 (DIAsource IL-10-ELISA Kit), Human TGF-beta ELISA-Multispecies TGF-beta kit) in the patient group at 0. 12. And it was studied from serum samples at week 48 and

[1] Meram Devlet Hastanesi. Enfeksiyon Hastalıkları ve Klinik Mikrobiyoloji Uzmanı, barisbalasar@yahoo.com ORCİD :0000-0002-2151-9835

[2] Doc. Dr., Meram Tıp Fakültesi Enfeksiyon Hastalıkları ve Klinik Mikrobiyoloji Uzmanı, drerayman@yahoo.com. ORCID:0000-0002-7491-2710

from a single serum sample in the control group. Values detected in serum samples were recorded as pg/ml. Serum ALT values in the patient and control groups were routinely studied in the biochemistry laboratory of our hospital. Combined treatment was started in the case group and the patients were evaluated in terms of response to the treatment at the 12th and 48th weeks. They were divided into 2 groups as responding and non-responsive to treatment. TNF-alpha, TGF-beta and IL-10 serum cytokine values of these two groups were 0-12 weeks, 12-48 weeks and 0-48. Pairwise comparisons were made within weeks. Finally, cytokine values at 0, 12, 48 weeks were compared with ALT. Spearman's correlation test was used for statistical analysis. The limit of significance was taken as $p<0.05$ in the interpretations. SPSS (version: 17.0) package program was used for statistical analysis. A statistical difference was found in serum TNF-alpha, TGF-beta and IL-10 serum cytokine values in patients who responded to treatment.

Key Words: Biological Markers; Liver Cirrhosis; Chronic hepatitis c; Cytokines

MATERIAL AND METHOD

The study was made in a single center. The location was Necmettin Erbakan University Meram Faculty of Medicine Hospital Infection Diseases and Clinical Microbiology Department. Consent was taken from Meram Faculty of Medicine Ethics Board (ethics board decree no 206, dated 2008) and from the patients and controls included in the study and all participants were informed in writing.

Patients and Controls

There were 43 individuals both in the patient and control groups. 23 of the individuals were male and 20 were female in both patient and control groups. Age median was 49.54±13.02 for the patient group and 47.98±14.1 for the control group. Diagnosis criteria determined by American Association for the Study of Liver Diseases (AASLD) were used for Chronic hepatitis C (CHC) diagnosis. Based on this, cases continuing to have anti-HCV and HCV-RNA positivity for more than six months were defined as CHC. CHC patients who didn't have any treatment before (naive) were included in the study. All patients were genotype 1b and were started pegile interferon and ribavirin combined treatment. Individuals who have at least one of chronic liver disease causes except chronic alcohol use, pregnancy and Hepatitis C virus (HCV) were excluded from the study. Patients who also have a severe systemic disease, use drugs which may cause hepatotoxic or liver steatosis, regularly take alcohol over 50 g/day, have clinically decompansated cirrhosis diagnosis and additional disease (HBsAg positivity, Anti-HIV positivity, hematological disease, autoimmune disease, congestive heart failure, chronic kidney failure, hematochromatosis) were not included in the study. Among CHC diagnosed patients, those who took antiviral and/or interferon treatment before were not included in the study. Individuals who didn't have the additional diseases we mentioned before and have negative anti-HCV were included in the control group.

Liver Biopsy

Liver biopsy was made for patients diagnosed with CHC before starting combined treatment. Liver biopsy wasn't made in the control group. ISHAK scoring providing separate evaluation for the degree and staging of liver biopsies was used. Biopsy results were evaluated by a pathologist. Biopsy score, aminotransferase and viral load values of the patients were evaluated in the same week to provide that they reflect pre-treatment period and to lower the margin of error in correlation tests.

Microbiological Analysis

Anti-HCV was studied with chemiluminescence method and vitrosEC (ortho-clinical Diagnostics) device using 3rd generation anti-HCV kits. Quantative HCV RNA determination (viral load) was made in Konya University Meram Faculty of Medicine Microbiology Department Serology Lab using Cobas Amplicor HCV Cobas Taqman (Roche diagnostic systems, USA) in patients detected to have anti-HCV positivity in serum. HCV RNAs of the patients were determined as IU/ml. Values below 50 IU/ml were regarded as negative. In HCV RNA positive patients, genotype study was made in a private laboratory using ABI PRISM 310 Genetic Analyzer (Perkin Elmer, USA) device.

Biochemical Analysis

Serum alanine aminotransferase (ALT), tumor necrosis factor alpha (TNF-alpha), tumor growth factor beta (TGF-beta), interleukin 10 (IL-10) values were studied in patient and control groups. 10 cc venous blood was taken in paraffin containing biochemistry tubes to measure TNF-alpha, TGF-beta and IL–10 values. Blood samples were centrifuged at 5000 rev for five minutes. These operations were completed within an hour. Serums taken in eppendorf tube after centrifuging were kept in deep freeze at -80 degrees until the study day. Serum cytokine levels were measured with ELISA method according to kit procedures. Human TNF-alpha (DIAsource TNF-alpha - ELISA Kit), Human IL–10 (DIAsource IL–10-ELISA Kit), Human TGF-beta ELISA-Multispecies TGF-beta kit)'levels were studied on serum samples in the 0., 12. and 48. weeks of the treatment in the patient group and on a single serum sample in the control group. Values detected in serum samples were registered in pg/ml. Serum ALT values were routinely studied in biochemistry laboratory in patient and control groups.

Statistical Analysis

The connection between liver fibrosis degree (stage) and histological activity index (HAI) scores and the TNF-alpha, TGF-beta and IL-10 values of the case and control groups was investigated. Then serum TNF-alpha, TGF-beta and IL-10 values in 0., 12. and 48. weeks and fibrosis and HAI scores were compared for the patients in the case group. Combined treatment was started in the case group and the patients were evaluated in the 12. and 48. weeks for treatment response. Patients were divided into two groups as responsive and irresponsive to treatment. Mutual comparisons were made on the 0-12., 12-48. and 0-48. weeks for TNF-alpha, TGF-beta and IL-10 serum cytokine values in these two groups. Finally, cytokine values and ALT were compared in the 0., 12. and 48. weeks. Spearman's correlation test was used for statistical analysis. Significance limit was taken $p<0.05$ in the interpretations. SPSS (version: 17.0) package program was used for statistical analyses.

FINDINGS

43 CHC patients and 43 control group participants were included in the study. Although the mean age was 49.54±13.02 for the patient group and 48.3±14.1 for the control group, the ages changed between 36 and 65 in the patient group and 34 and 63 in the control group. No statistical difference was observed among the two groups. 23 individuals were male and 20 were female in both patient and control groups.

Data for case and control groups and minimum, maximum and average values of TNF-alpha, TGF-beta and IL-10 levels for case and control groups can be seen on Table-1 below.

Table-1: IL-10, TNF-alpha and TGF-beta values detected in the 0. week in patient and control groups are given on the table.

	Case (n:43)			Control (n:43)		
	Minimum pg/ml	**Maximum pg/ml**	**Average pg/ml**	**Minimum pg/ml**	**Maximum pg/ml**	**Average pg/ml**
IL-10 (0)	30.0	66.0	43.3	0.0	19.0	6.8
TNF-alpha (0)	30.0	550.0	318.5	0.0	23.0	6.5
TGF-beta (0)	6000.0	140000.0	14159.7	1000.0	10000.0	3267.4

Investigating the connection between Stage and HAI scores and TNF-alpha, IL-10 and TGF-beta values of case and control groups

IL-10, TNF-alpha and TGF-beta values detected in patient and control groups are given on the table. IL-10, TNF-alpha and TGF-beta serum levels of the patient group at the zeroth week meaning the beginning of treatment were compared to IL-10, TNF-alpha and TGF-beta serum levels of the control group.

While IL10 (0) average value was 6.8 pg/ml in the control group, the approximate value found in the case group was 43.3 pg/ml which was nearly seven times higher than this value. There was a statistically significant difference among the two groups (P:0.000).

TNF-alpha (0) was compared in case and control groups. While average TNF-alpha value was 6 pg/ml in the control group, it was 318 pg/ml in the case group. A statistically significant difference was detected among them (p:0.000).

TGF-beta (0) was compared in case and control groups. While average TGF-beta was 3267 pg/ml in the control group, it was 14159 pg/ml in the case group which was found to be statistically significant (p:0.000). When the levels of the three cytokines were evaluated, a statistically significant difference was found between the patient and control groups ($p<0.05$).

Classification and Statistical Results of the Case Group Based on Treatment Responses in 12th and 48th weeks

Patients in the case group were separated into two groups as those with and without early virological response (EVR) in 12th week. All patients without EVR also didn't have end of treatment response (ETR). There was no treatment response in 9 of 43 cases. Case group in Table-2 was separated into two groups as responsive and irresponsive to treatment and together with control group, patient numbers were provided.

Table 2. Classification of Case and Control Groups. There was no treatment response in 9 of 43 cases in the case group.

Groups	• **week**	**12th week**	**48th week**
No response to treatment		**9**	**9**
Response to treatment		**34**	**34**
Control	**43**		

IL-10, TNF-alpha and TGF-beta serum cytokine values of the patients separated into two groups as responsive and irresponsive to treatment were compared among themselves in the 0.-12., 12.-48. and 0.-48. weeks. Average cytokine values and statistical p values are given in the table below. P<0.05 is statistically significant.

Table 3. Patients in the case group were separated into two groups as responsive and irresponsive to treatment. **IL-10, TNF-alpha and TGF-beta average cytokine values of both groups in the 0., 12. and 48. weeks can be seen.**

Mean cytokine values of patients in case group (pg/ml)		
	No response to treatment	Response to treatment
IL-10 (0)	43	43.41
IL-10 (12th week)	34.66	38.61
IL-10 (48th week)	22.66	27.35
TNF-alpha (0)	367	305
TNF-alpha (12th week)	365.33	289.55
TNF-alpha (48th week)	355.22	268.35
TGF-beta (0)	59905	22260
TGF-beta (12th week)	53113.33	32004.41
TGF-beta (48th week)	55088.88	29553.29

Table 4. p values for L-10, TNF-alpha and TGF-beta serum cytokine and ALT values compared inn 0-12, 12-48 and 0-48. weeks.

Statistical p values		
Comparative serum cytokine and ALT values	No response to treatment	Response to treatment
IL-10 (0-12th week)	0.012	0.0
IL-10 (12-48th week)	0.011	0.0
IL-10 (0-48th week)	0.011	0.0
TNF-alpha (0-12th week)	**0.058**	0.0
TNF-alpha (12-48th week)	**0.075**	0.001
TNF-alpha (0-48th week)	**0.091**	0.0
TGF-beta (0-12th week)	**0.055**	0.001
TGF-beta (12-48th week)	**0.066**	0.001
TGF-beta (0-48th week)	**0.074**	0.0
ALT (0-12th week)	**0.054**	0.0
ALT (12-48th week)	**0.588**	0.003
ALT (0-48th week)	**0.097**	0.003

In IL-10 serum cytokine value comparisons in 0-12, 12-48 and 0-48th weeks, a statistical significance was found both in treatment responsive and irresponsive groups. Regardless of treatment response, a decrease was observed in serum IL-10 values in the 12. and 48. weeks compared to the beginning in both groups. This decrease was higher in treatment irresponsive group compared to the responsive group. Although there is a decrease in serum IL-10 values in EVR and ETR patients, it is less compared to irresponsive patients.

The condition is a little bit different in serum TNF-alpha and TGF-beta values. While high serum TNF-alpha and TGF-beta cytokine values continue in treatment irresponsive group, there was a gradual decrease in treatment responsive group. In patients responsive to treatment, the decrease in serum IL-10, TNF-alpha and TGF-beta cytokine values in both 12. and 48. weeks was statistically significant. The condition is the same for serum ALT values. While there was a gradual decrease in serum ALT values in treatment responsive group, the values remained high in treatment irresponsive groups. This condition shows the correlation between serum TNF-alpha and TGF-beta and ALT. Serum cytokine values and ALT levels were compared to detect the presence of such a correlation and its level its level. This comparison is covered below.

Comparison of serum IL-10, TNF-alpha and TGF-beta cytokine levels and ALT in 0-12-48. weeks

Patients in the case group were separated into two groups as responsive and irresponsive to treatment. Serum IL-10, TNF-alpha and TGF-beta cytokine levels and ALT values in 0., 12. and 48th weeks were compared in both groups. In the comparisons made, a positive correlation was found among the serum cytokine values and ALT in 0., 12. and 48. weeks in patients responsive to treatment. With the decrease in serum cytokine values in 0., 12. and 48. weeks in patients responsive to treatment, a gradual decrease was also detected in ALT values. There is a linear connection between cytokine values and ALT in patients responsive to treatment.

The situation is a little bit different in patients irresponsive to treatment. While a positive correlation is observed among serum TNF-alpha and TGF-beta cytokine

values in these patients, there was a negative correlation among serum IL-10 value and ALT. This situation is statistically significant. The most significant connection was between TNF-alpha and ALT. In addition to serum TNF-alpha and TGF-beta cytokine values in 0., 12. and 48. weeks in patients irresponsive to treatment, ALT values also remained high. A completely opposite condition was detected for IL-10. While serum IL-10 values significantly decrease in time in patients irresponsive to treatment, ALT values remain high.

DISCUSSION AND CONCLUSION

Cellular immune response has a central role in liver disease pathogenesis in CHC patients. The imbalance between pro- and anti-inflammatory cytokines causes the advancement of cytolysis and/or hepatic lesions or fibrosis (13). Immune response effective for hepatitis virus is provided by Ts (CD8+) and Th (CD4+) cells. It is considered that B lymphocytes regulate the cytokine release and the B and CD8+ T cell activity of these cytokines by presenting MHC class II molecules and viral peptides to CD4+ T lymphocytes (2-3).

HBV and HCVs intervene the cytokine network at different levels and inhibit Th2/Tc2 cytokine profile and thus flee the immune response. Inadequacy in infection control mechanism causes the constant infiltrations of inflammatory cells induced by pro-inflammatory chemokines into the liver parenchyma and liver damage and as a result, liver cirrhosis develops (16).

Progression of HCV infected patients to fibrosis has not been completely understood. Early response of Ts (CD8+) cells may be important in viral clearance (17). Many cytokines and chemokines play a role in antiviral mechanism. TNF-alpha, TGF-beta and IL–10 are some of these. Acute and chronic liver diseases constitute an inflammatory phase related to the increased expression of pro and anti-inflammatory cytokines (20). In this regard, different cytokine responses formed against the virus cause a different liver damage (21).

In our study, TNF-alpha, TGF-beta and IL-10 among the cytokines which can be effective against liver inflammation and fibrosis were investigated. TNF-alpha is the first of these. TNF-alpha (12) which is the main mediator of acute inflammatory response caused by infectious pathogens provides the inflammation progression by inducing the defense mechanism (6,14). Inducing antibody production, TNF-alpha may also cause the exacerbation of the infection (17). TNF-alpha is produced and secreted in the mononuclear cells infiltrating in local inflammatory areas of the liver and this makes us to consider that TNF-alpha may play a role in the inflammatory activity in chronic liver disease.

In our study, serum TNF-alpha levels were compared in case and control groups. Serum TGF-alpha values were found higher in HCV-infected patients compared to the control group. Similarly, in the study by Zylberg et al. (12) on 60 CHC patients, TNF-alpha level was found higher and statistically significant in patients with CHC infection compared to the healthy control group. The fact that TNF-alpha is a cytokine playing a role in inflammatory process explains this condition.

The inflammation demonstrator in liver is determined with HAI in HCV patients. There is a significant connection between HAI and ALT levels. ALT values increasing parallel to HAI point out. To determine the connection between TNF-alpha and HAI, TNF-alpha values and HAI degrees of the patients in the case group were compared in our study. Regardless of the fibrosis score, TNF-alpha was found significantly higher in patients with average degree of HAI compared to those with minimal HAI. Neuman et al (17) also reached the same result. In their study including 778 CHC patients, Benhamou et al (18) detected a positive correlation between liver inflammation degree and TNF-alpha levels (rho:0.92).

The connection between TNF-alpha serum levels and fibrosis scores was also investigated in our study. Serum TNF-alpha values were detected significantly high in cirrhosis patients compared to patients with mild fibrosis or no fibrosis. Highest TNF-alpha values were observed in cirrhosis and active CHC patients. Many other studies support our study. (2,4,8,12).

In a study made by Maria et al (145) TNF-alpha values before the treatment were measured three times higher in CHC patients compared to the control group and this was found to be statistically significant. They were measured 2.2 times higher in patients without EVR. 1.2 was the value found in patients with EVR, which is a very low value. The highest TNF-alpha values were measured in pre-treatment period in this study. They were found at very low values in patients with EVR. TNF-alpha values in 0-12., 12-48. and 0-48. weeks were compared in patients responsive and irresponsive to treatment were compared among themselves in our study.

TNF-alpha gradually decreased in patients responsive to treatment. In patients responsive to treatment, lowest TNF-alpha values were observed in 48th week. This linear connection was found to be statistically significant. Although TNF-alpha values in the 12. week were lower compared to the values at the beginning, they were also statistically significant. TNF-alpha remained high in patients irresponsive to treatment. A difference wasn't found in TNF-alpha values in 0., 12. and 48. weeks in patients irresponsive to treatment.

TNF-alpha and ALT values in 0., 12. and 48. weeks were also compared in our study and a positive correlation was found. In patients responsive to treatment, a gradual decrease was observed in serum ALT values in addition to TNF-alpha values. This situation is statistically significant. Starting from the relation found between HAI and TNF-alpha at the beginning of the treatment, we think that the continuing high values show that the inflammation continues.

The other important cytokine, TGF-beta, inhibits hepatocyte proliferation in liver regeneration and strongly stimulates the production of extracellular matrix proteins by hepatocyte in liver cirrhosis. It plays a significant role in fibrosis pathogenesis in TGF-beta, chronic hepatitis and cirrhosis. In our study, TGF-beta values were compared in case and control groups. TGF-beta values were found higher in HCV-infected patients compared to the control group. It is stated that TGF-beta reflects the histological stage and TGF-beta activation is the starting point of fibrogenesis (12). In a study by Kırmaz et al (14), serum TGF-beta values were found higher in CHC patients compared to the control group. TGF-beta values and fibrosis scores of patients in case group were also compared in our study. Serum TGF-beta values were found significantly higher in cirrhosis patients compared to patients with mild fibrosis or no fibrosis. As the result of the study made by Neuman et al (18), TGF-beta values were found to be low compared to patients with mild fibrosis (F2-3) compared to patients with minimal fibrosis (F0-1), regardless of the HAIs and this was regarded to be statistically significant. In the study by Li H (6), a positive correlation was found between fibrosis severity and TGF-beta values. But TGF-beta values significantly decreased in patients with severe fibrosis. Similar findings were acquired in our study. TGF-beta values of stage 5 and stage 6 patients were found lower compared to stage 3 and 4 patients in our study. A statistical study couldn't be made due to inadequate number of patients in 5th and 6th stages.

Different from other studies, TGF-beta values in 0-12., 12-48. and 0-48. weeks were compared among themselves in patients responsive and irresponsive to treatment in the studies. TGF-beta values gradually decreased in patients responsive to treatment. TGF-beta values remain high in patients irresponsive to treatment. TGF-beta and ALT values in 0., 12. and 48. weeks were also compared in our study and a positive correlation was found. Presence of a linear connection between TGF-beta and HAI and HAI and ALT values at the beginning of the treatment, correlated decrease in TGF-beta values in patients with decreasing ALT values with treatment made us consider that a prediction can be made on HAI looking at the TGF-beta levels in patients responsive to treatment.

IL–10 is the cytokine released by macrophages and it inhibits their functions. IL-10 also known as cytokine secretory inhibitor factor has an anti-inflammatory effect. It normalized the ALT level with its anti-inflammatory effect, limits hepatic lesions and lowers fibrosis. IFN-gama synthesis is inhibited by IL-10 (12). In our study, serum IL-10 values were compared in case and control group. Average serum IL-10 values were found to be nearly seven times higher in the case group compared to the control group. In the study by Moore et al (23), IL-10 values were found higher than the control group in CHC patients. Ayramescu et al (15) and Abaylı et al (16) found IL-10 values to be higher in CHC patients compared to the healthy control group. A statistical difference wasn't found in IL-10 values between HAI and stage in our study. Like TNF-alpha and TGF-beta values, IL-10 values were found lower in patients responsive to treatment. While TNF-alpha and TGF-beta values remained high in patients irresponsive to treatment, the condition is a little bit different for IL-10. The decrease in IL-10 values was higher in treatment irresponsive group compared to the responsive group. While there was a decrease in serum-IL-10 values especially in EVR and ETR patients, it was less compared to irresponsive patients.

Parallel to high TNF-alpha and TGF-beta serum cytokine values, we mentioned before that ALT values remained high in patients irresponsive to treatment in our study. A completely opposite condition was detected for IL-10 values. While serum IL-10 values significantly decrease in time in patients irresponsive to treatment, ALT values remain high. In a study by Nelson et al (22), subcutaneous IL-10 was given to 30 patients diagnosed with severe fibrosis and had unsuccessful antiviral treatment. This caused an important and significant recovery in serum ALT values. In latter control, a decrease was noticed in hepatic inflammation score in 13 and in fibrosis score in 11 of 28 patients (23). On the other hand, serum HCV RNA levels increased 0.5 logs in the same patients during the treatment. As a result, while IL-10 has a significant role in the decreasing of hepatic inflammation, it also causes inflammations in chronic hepatitides as the immune system is suppressed. This situation can explain decreasing IL-10 levels, high ALT values and inflammations. In our study, there was an inverse correlation between IL-10 values and ALT values. IL-10 is a cytokine with a very complicated in vivo anti-inflammatory effect. IL–10 is a useful cytokine as long as it is in balance with proinflammatory cytokines. The irregularity in cytokine balance due to chronic hepatitides harms the functionality of the cytokines. The higher decrease in serum IL-10 values in patients irresponsive to treatment compared to the responsive ones may explain this condition. Decreasing of anti-inflammatory cytokines such as IL-10 in irresponsive patients causes the sliding of the balance towards proinflammatory side and this condition is shown with hepatic inflammation and thus high ALT.

As a result, there is a linear connection between liver HAI and ALT. There is a linear connection also between Liver HAI and cytokines. In patients responsive to treatment, there is a gradual decrease in cytokine and ALT values. We can predict that HAI would indirectly decrease in this situation. Similarly, there was a linear decrease in ALT values together with the cytokines in patients responsive to treatment. Mutual evaluation of serum cytokine and ALT values may help us to predict HAI progression.

REFERENCES

1.Tanaka J., Akita T., Ohisa M. Sakamune K., Ko K., Uchida S. et al. Trends in the total numbers of HBV and HCV carriers in Japan from 2000 to 2011. Journal of Viral Hepatitis 2018; 25: 363-72.

2.Kang W., Shin E. Clinical Implications of Chemokines in Acute and Chronic Hepatitis C Virus Infection. Yonsei Med. J 2011; 52: 871-78.

3.Reiman RM, Thompson RW, Feng CG, Hari D, Knight R, Cheever AW, et al. Interleukin–5 (IL–5) augments the progression of liver fibrosis by regulating IL–13 activity. Infect Immun 2006;74(3):1471–1479.

4.Knop V., Hofmann W.P., Buggisch P., Klinker H., Mauss S., Gunther R. et al. Estimation of liver fibrosis by noncommercial serum markers in comparison with transient elastography in patients with chronic hepatitis C virus infection receiving direct-acting antiviral treatment. Journal of Viral Hepatitis 2019; 26: 224-30.

5.Chen S.L., Morgan T.R. The natural history of hepatitis C virus (HCV) infection. Int. J. Med. Sci 2006; 3: 47–52.

6.Li H., Huang M.H., Jiang J.D., Peng Z.G. Hepatitis C: From inflammatory pathogenesis to anti-inflammatory hepatoprotective therapy. World J. Gastroenterol 2018; 24: 5297–311.

7.Persico M, Persico E, Suozzo R, Conte S, De Seta M, Coppola L, et al. Natural history of hepatitis C virus carriers with persistently normal aminotransferase levels. Gastroenterology 2000; 118: 760–64.

8.Fallhi P, Ferrari SM, Giuggioli D, Sebastiani M, Colaci M, Ferri C, et al. Chemokines in the Pathogenesis and as Therapeutical Markers and Targets of HCV Chronic Infection and HCV Extrahepatic Manifestations. Curr. Drug. Targets 2017; 18: 786–93.

9.Kronenberger B, Welsch C, Forestier N, Zeuzem S. Novel Hepatitis C Drugs in Current Trials. Clin. Liver Dis 2008; 12: 529–55.

10.Perlin Cm, Ferreira VL, Borba HHL, Wiens A, Ivantes CAP, Lenzi L, et al. Quality of life in Brazilian patients with treated or untreated chronic hepatitis C. Rev. Inst. Med. Trop. Sao Paulo 2017; 59: 81.

11.Post J, Ratnarajah S, Lloyd AR Immunological determinants of the outcomes from primary hepatitis C infection. Cell. Mol. Life. Sci 2009; 66: 733–56.

12.Zylberberg H, Rimaniol AC. Pol S, Masson A, De Groote D, Berthelot P. et al. Solubl tumor necrosis factor receptors in chronic hepatitis C: a correlation with histological fibrosis and activity. J. Hepatol 1999; 30: 185–91.

13.Reiman RM, Thompson RW, Feng CG, Hari D, Knight R, Cheever AW, et al. Interleukin–5 (IL–5) augments the progression of liver fibrosis by regulating IL–13 activity. Infect Immun 2006;74(3):1471–1479.

14.Kirmaz C, Terzioglu E, Topalak O, Bayrak P, Yilmaz O, Ersoz G, et al. Serum transforming growth factor-beta1 (TGF-beta1) in patients with cirrhosis, chronic hepatitis B and chronic hepatitis C. Eur. cytokine netw 2004; 15: 112-16.

15.Avramescu CS, Comanescu V, Popescu SN, Turculeanu A, Balasoiu M, Popescu CF, et al. Correlations among the serum levels of some interleukins and the histopathological aspects in chronic viral hepatitis C. Rom. J. Morphol. Embryol 2008; 49: 57–62.

16.Abayli B, Canataroglu A, Akkiz H. Serum profile of T helper 1 and T helper 2 cytokines in patients with chronic hepatitis C virus infection. Turk J. Gastroenterol 2003; 14: 7–11.

17.Neuman MG, Benhamou JP, Malkiewicz IM, Ibrahim A, Valla DC, Martinot- Peignoux M, et al. Kinetics of serum cytokines reflect changes in the severity of chronic hepatitis C presenting minimal fibrosis. J. Viral Hepat 2002; 9: 134-40.

18.Neuman MG, Benhamou JP, Marcellin P, Valla D, Malkiewicz IM, Katz GG, et al. Cytokine—chemokine and apoptotic signatures in patients with hepatitis C. Transl. Res 2007; 149: 126-36.

19.Mohamadnejad M, Montazeri G, Fazlollahi A, Zamani F, Nasiri J, Nobakht H, et al. Noninvasive markers of liver fibrosis and inflammation in chronic hepatitis B-virus related liver disease. Am. J. Gastroenterol 2006; 101: 2537–545

20.Abbas Z, Moatter T, Hussainy A, Jafri W. Effect of cytokine gene polymorphism on histological activity index, viral load and response to treatment in patients with chronic hepatitis C genotype 3. World J Gastroenterol. 2005 Nov 14;11(42):6656–61.

21.Yoshioko K, Kakumu S, Arao M, Tsutsumi Y, İnoue M, Wakita T et al. Immunohistochemical studies of intrahepatic tumor necrosis factor alpha in chronic liver disease. J Clin Pathol 1990;43.298–302.

22.Nelson DR, Tu Z, Soldevila-Pico C, Abdelmalek M, Zhu H, Xu YL, et al. Long-term interleukin 10 therapy in chronic hepatitis C patients has a proviral and anti-inflammatory effect. Hepatology. 2003 Oct;38(4):859–68.

23.Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin–10 and the interleukin–10 receptor. Annu Rev Immunol. 2001;19.683–765.