

Inmunología

www.elsevier.es/inmunologia



Original article

Evaluation of serum levels of IL-6, TNF- α , IL-10, IL-2 and IL-4 in patients with chronic hepatitis



Antonia Mourtzikou^{a,b}, Maria Alepaki^a, Marilena Stamouli^{c,*}, Abraham Pouliakis^a, Anastasios Skliris^c, Petros Karakitsos^a

^a Department of Cytopathology, Attikon University Hospital, Athens, Greece

^b Department of Biochemistry, Asklepieio Voulas General Hospital, Athens, Greece

^c Department of Biochemistry, Naval and Veterans Hospital, Athens, Greece

ARTICLE INFO

Article history:

Received 9 September 2013

Accepted 14 January 2014

Available online 12 March 2014

Keywords:

Hepatitis B

Hepatitis C

Cytokines

Serum levels

ABSTRACT

Background: Changes in various cytokine activities have been reported during both HBV and HCV infections, while an imbalance of pro-inflammatory and anti-inflammatory cytokine production influences their immunopathogenesis. The aims of the present study are (a) to measure serum levels of interleukin-6 (IL-6), tumor necrosis factor α (TNF- α), interleukin-10 (IL-10), interleukin-2 (IL-2) and interleukin-4 (IL-4) in a sample of patients affected either by chronic HBV infection or by chronic HCV infection and in healthy controls (b) to correlate serum levels of IL-6, TNF- α , IL-10, IL-2 and IL-4 with biochemical markers of liver disease and (c) to evaluate differences of the aforementioned cytokines between HBV and HCV patients, as well as between patients and healthy controls.

Methods: The study population consisted of 50 patients with chronic hepatitis B, 40 patients with chronic hepatitis C and 30 healthy controls aged between 28 and 75 years. Biochemical markers of liver disease were evaluated by routine methods approved by IFCC. Serum concentrations of IL-6, TNF- α , IL-10, IL-2 and IL-4 were determined with the Human Cytokine/Chemokine Panel I Merck Millipore.

Results: HBV patients showed statistically significant difference in TNF- α and IL-2 levels, versus healthy controls. HCV patients showed statistically significant difference in TNF- α , IL-10 and IL-2 levels versus healthy controls. IL10 and IL-2 levels were significantly different between HBV and HCV patients.

Conclusions: This study evaluated the serum cytokine levels (IL-6, TNF- α , IL-10, IL-2 and IL-4) of chronic hepatitis B or C patients, as well as the differences in such levels between patients and healthy controls. Correlations of cytokine levels with biochemical markers of liver disease were also observed, reflecting the degree of activity of the inflammatory process in the liver.

© 2013 Sociedad Española de Inmunología. Published by Elsevier España, S.L. All rights reserved.

* Corresponding author.

E-mail addresses: marilena-stamouli@yahoo.com, mixalodimitrakis@yahoo.com (M. Stamouli).

0213-9626/\$ – see front matter © 2013 Sociedad Española de Inmunología. Published by Elsevier España, S.L. All rights reserved.

<http://dx.doi.org/10.1016/j.inmuno.2014.01.001>

Evaluación de las concentraciones séricas de IL-6, TNF- α , IL-10, IL-2 e IL-4 en pacientes con hepatitis crónica

R E S U M E N

Palabras clave:

Hepatitis B
Hepatitis C
Citocinas
Concentraciones séricas

Antecedentes: Tanto en las infecciones por VHB como por VHC se han notificado cambios en las diversas actividades de las citocinas, y es conocido que su inmunopatogénesis está influida por un desequilibrio en la producción de citocinas pro y antiinflamatorias. Los objetivos del presente estudio son: a) medir las concentraciones séricas de interleucina 6 (IL-6), factor de necrosis tumoral α (TNF- α), interleucina 10 (IL-10), interleucina 2 (IL-2) e interleucina 4 (IL-4) en una muestra de pacientes afectados, ya sea por una infección crónica por VHB o por VHC y en controles sanos; b) correlacionar las concentraciones séricas de IL-6, TNF- α , IL-10, IL-2 e IL-4 con los marcadores bioquímicos de enfermedad hepática, y c) evaluar las diferencias de las citocinas antes mencionadas entre los pacientes con VHB y con HCV, así como entre los pacientes y los controles sanos.

Métodos: La población del estudio consistió en 50 pacientes con hepatitis B crónica, 40 pacientes con hepatitis C crónica y 30 controles sanos de edades comprendidas entre 28 y 75 años. Se evaluaron los marcadores bioquímicos de la enfermedad hepática mediante métodos rutinarios aprobados por la IFCC. Las concentraciones séricas de IL-6, TNF- α , IL-10, IL-2 e IL-4 se determinaron mediante el Panel I de citocinas/quimiocinas humanas de Merck Millipore.

Resultados: Los pacientes con VHB mostraron diferencias estadísticamente significativas en las concentraciones de TNF- α e IL-2, comparadas con las de controles sanos. Los pacientes con HCV mostraron diferencias estadísticamente significativas en TNF- α , IL-10 e IL-2 respecto a las concentraciones de los controles sanos. Las concentraciones de IL-10 e IL-2 fueron significativamente diferentes entre los pacientes con VHB y con HCV.

Conclusiones: Este estudio evaluó las concentraciones séricas de citocinas (IL-6, TNF- α , IL-10, IL-2 e IL-4) de pacientes con hepatitis crónica B o C, así como las diferencias en dichas concentraciones entre pacientes y controles sanos. También se observaron correlaciones de las concentraciones de citocinas con marcadores bioquímicos de la enfermedad hepática, lo que refleja el grado de actividad del proceso inflamatorio en el hígado.

© 2013 Sociedad Española de Inmunología. Publicado por Elsevier España, S.L. Todos los derechos reservados.

Introduction

Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections constitute serious global health problems due to their high morbidity and mortality. It is estimated that 2 billion people worldwide have been infected with HBV, among which 350 million are chronically infected.¹⁻³ It is also estimated that about 200 million people are infected with HCV, among which 170 million are chronically infected.^{4,5} Chronic HBV and HCV infections can have severe consequences including debilitating symptoms, impaired quality of life, disability, costly health care expenditures and death. More than 600,000 deaths are attributed to HBV infection annually, while more than 350,000 deaths are attributed to HCV infection annually. Most of these deaths are caused by liver cirrhosis and hepatocellular carcinoma.^{2,6-8} However, the pathogenesis of liver damage during chronic HBV and HCV infections is not fully understood. Although HBV and HCV are both hepatotropic viruses, they differ in the way they interact with the host immune system. In particular, HBV establishes chronic infection mainly by infecting neonates, but it is controlled by most newly infected adults. On the other hand, in the case of HCV, chronic infection occurs in 75% to 85% of the infected individuals.⁹ The reasons for viral persistence of these two viruses and transformation

from acute to chronic infection are not clear, but it is known that both viral and host characteristics can influence the outcome of the infection.^{9,10} The host response to hepatitis viruses involves various components of the immune system, including T-lymphocyte immune-regulatory cytokines. Cytokines are a group of protein molecules involved in various biological processes including growth, differentiation, cell survival, hematopoiesis, immunological functions, inflammation, apoptosis, necrosis and fibrosis.^{11,12} The cytokine group is highly heterogeneous and consists of different types of molecules, such as the interleukins, the tumor necrosis factor family, the interferons, the chemokines, the transforming growth factor- β and others. The control of cytokine production is highly complex, while the effects of cytokines are widespread throughout multiple regulatory molecule networks. Cytokines are produced by a wide variety of cells, mainly the Th1 and Th2 cells. Th1 cells secrete pro-inflammatory cytokines, whereas the Th2 cells secrete anti-inflammatory cytokines. Th1 cytokines are involved in cell-mediated immunity, and play a crucial role in protection from intracellular pathogens and are associated with recovery. Th2 cytokines regulate humoral immune responses, and their rising levels are often associated with the development of persistent infections.¹¹⁻¹³ The liver is a major organ in the production of cytokines. Cytokines are involved in physiologic

and pathologic processes occurring in the liver. They play an important role in liver growth and regeneration, as well as in inflammatory processes including viral liver disease, in liver fibrosis and cirrhosis.^{11,12} They can recognize virus-infected cells and can regulate the immunological and inflammatory responses, viral clearance and tissue damage mechanisms. Changes in various cytokine activities have been reported during both HBV and HCV infections, while an imbalance of pro-inflammatory and anti-inflammatory cytokine production influences their immunopathogenesis.^{9,13} In particular, alterations in serum and intrahepatic Th1 and Th2 cytokine patterns play a critical role in viral persistence, as well as in host immune response, in liver damage and liver disease progression from chronic hepatitis to hepatocellular carcinoma.¹⁴⁻¹⁷ Given that cytokines are involved in many processes occurring in the liver which impact on the development of chronic hepatitis, it is of interest to study their levels in chronic hepatitis patients and their correlation with markers of liver disease.

The aims of the present study are (a) to measure serum levels of interleukin-6 (IL-6), tumor necrosis factor α (TNF- α), interleukin-10 (IL-10), interleukin-2 (IL-2) and interleukin-4 (IL-4) in a sample of patients affected either by chronic HBV infection or by chronic HCV infection and in healthy controls (b) to correlate serum levels of IL-6, TNF- α , IL-10, IL-2 and IL-4 with biochemical markers of liver disease and (c) to evaluate differences of the aforementioned cytokines between HBV and HCV patients as well as between patients and healthy controls.

Materials and methods

The study population consisted of 50 patients with chronic hepatitis B (27 men and 23 women), 40 patients with chronic hepatitis C (32 men and 8 women) and 30 healthy controls (15 men and 15 women) aged between 28 and 75 years (mean age 49.2 years). Patients were recruited from the Internal Medicine Department, of Asklepíeio Voulas General Hospital, during a three-year period of time. Patients with chronic hepatitis B were HBsAg (+), anti-HBc (+), anti-HBe (+), HBVDNA (+) by PCR, anti-HCV (–) and anti-HDV (–). Patients with chronic hepatitis C were anti-HCV (+), HCV RNA (+) by PCR, HBsAg (–), anti-HBc (–) and anti-HDV (–). All patients had liver histology consistent with chronic hepatitis. Presence of co-infection with human immunodeficiency virus was excluded in all patients in this study. Exclusion criteria also included co-existence of other causes of chronic liver disease, alcohol consumption and a history of immunosuppressive or antiviral therapy. The controls incorporated in the study were blood donors without any clinical history of hepatitis and were considered healthy through routine laboratory analysis.

Blood samples were collected by venipuncture and allowed to clot at room temperature for 30 min. After centrifugation for 10 min at $3000 \times g$ the sera were separated, aliquoted and then frozen at -70°C . Freeze/thaw cycles of the samples were completely avoided. When assayed, samples were thawed completely, mixed well by vortex and centrifuged to remove particulates. The study was approved by the Medical Ethics Committee of Attikon University Hospital and was conformant to the ethical guidelines of the Declaration of Helsinki.

Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT) and total bilirubin levels were measured on Siemens ADVIA 1800 fully automated analyzer (Siemens, Erlangen, Germany) at the Biochemistry Laboratory of Asklepíeio Voulas General Hospital. Measurements were performed by routine methods approved by IFCC. The upper limit of ALT activity was set at 35 IU/L, of AST at 37 IU/L, of ALP at 104 IU/L, of GGT 38 IU/L and of total bilirubin at 1.00 mg/dL.

Serum concentrations of IL-6, TNF- α , IL-10, IL-2 and IL-4 were determined with the Human Cytokine/Chemokine Panel I Merck Millipore (Cat. No. MPXHCYTO-60K, Millipore Corporation, Billerica), according to the manufacturer's instructions on the Luminex fully automated analyzer. Luminex uses a bead-based technology whose principles are as follows: using proprietary techniques it internally color-codes microspheres with two fluorescent dyes. Through precise selection of the concentrations of these dyes, distinctly colored bead sets can be created, each of which is coated with a specific capture antibody. After an analyte from a test sample is captured by the bead, a biotinylated detection antibody is introduced. The reaction mixture is then incubated with streptavidin-PE conjugate, serving as the reporter molecule, to complete the reaction on the surface on each microsphere. The microspheres are allowed to pass rapidly through a laser that excites the internal dyes marking the microsphere set. A second laser excites PE, i.e. the fluorescent dye on the reporter molecule. Finally, high-speed digital-signals identify each individual microsphere and quantify the result of its bioassay based on fluorescent reporter signals.

The assay procedure was given briefly as follows: The filter plate was prewashed with 200 μL Wash buffer on a plate shaker for 10 min at room temperature. Wash buffer was removed by vacuum and 25 μL of standards, controls, assay buffer, matrix solution and serum sample were added into the appropriate wells. 25 μL of the beads were added to each well. The plate was incubated with agitation overnight (for 16–18 h) at 4°C . After several washes, 25 μL of detection antibodies were added and were incubated for 1 h at room temperature followed by the addition of 25 μL streptavidin-phycoerythrin and 30 min incubation. Finally 150 μL of sheath fluid were added to each well and the beads were resuspended for 5 min on a plate shaker. The analysis was performed on the Luminex 200™ platform (Luminex Corporation). IL-6, TNF- α , IL-10, IL-2 and IL-4 serum concentrations were automatically calculated from standard curves. These concentrations were measured in duplicate in all patients. Serum concentrations of IL-6, TNF- α , IL-10, IL-2 and IL-4 were measured at the Department of Cytopathology of Attikon University Hospital being EN ISO 9001:2000 certified.

Statistical analysis

Correlations between pairs of variables were studied by linear regression analysis. Pearson correlation (r) was measured to estimate correlation between pairs comprising: two different cytokines (all possible pairs) and biochemical parameters, two different groups of patients (all possible pairs). The differences between HBV and HCV patients and the differences between

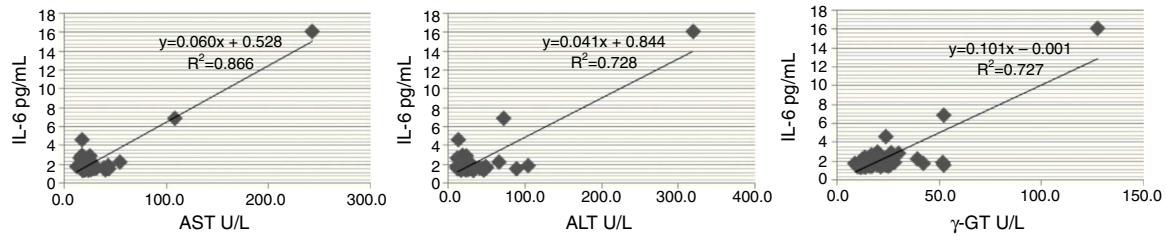


Fig. 1 – Relationship between serum AST, ALT and GGT and circulating IL-6 in HBV patients.

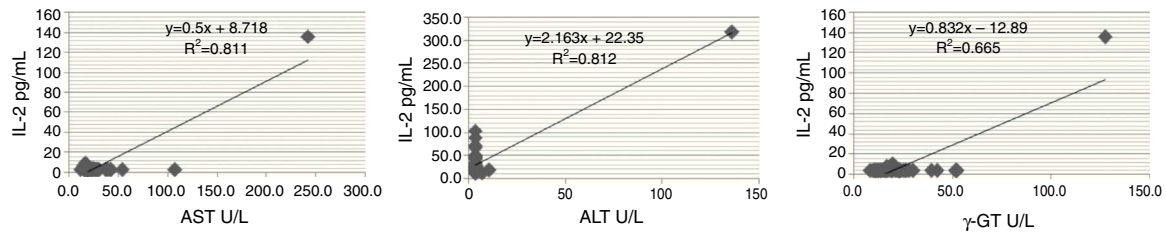


Fig. 2 – Relationship between serum AST, ALT, GGT and circulating IL-2 in HBV patients.

patients and healthy controls were analyzed by means of hypothesis testing for the equality or not of the means. They were evaluated by means of Student t-test, and p values lower than 0.05 were considered significant.

Results

Healthy controls

In healthy controls mean values were IL-6=0.744, TNF- α =1.323, IL-10=2.130, IL-2=1.668 and IL-4=1.765 pg/mL respectively.

HBV patients

AST levels ranged from 11.6 to 242.4 U/L (mean value 34.0), ALT levels ranged from 11.9 to 319.1 U/L (mean value 41.2), ALP levels ranged from 36.4 to 116.5 U/L (mean value 64.0), GGT levels ranged from 8.7 to 127.3 U/L (mean value 24.9) and total bilirubin levels ranged from 0.35 to 10.77 mg/dL (mean value 1.11). IL-6 levels ranged from 1.433 to 16.123 pg/mL (mean 2.287). High positive correlation was observed between IL-6 levels on the one hand and AST ($r=0.900$), ALT ($r=0.918$) and GGT ($r=0.853$) on the other hand (Fig. 1). TNF- α levels ranged from 1.522 to 46.944 pg/mL (mean 7.112). We did not observe any correlation between TNF- α levels and any of the biochemical

markers. IL-10 levels were lower than 2.600 pg/mL in all HBV patients. IL-2 levels ranged from 3.200 to 136.107 pg/mL (mean 8.542). High positive correlation was observed between IL-2 levels on the one hand and AST ($r=0.908$), ALT ($r=0.901$) and GGT ($r=0.815$) on the other hand (Fig. 2). IL-4 levels ranged from 3.200 to 49.732 pg/mL (mean 5.765). Positive correlation was observed between IL-4 levels and AST ($r=0.512$), ALT ($r=0.515$) and GGT ($r=0.484$) (Fig. 3).

Mean IL-6 and IL-4 levels were different in HBV patients than in healthy controls but these differences were not statistically significant. Statistically significant differences between HBV patients and healthy controls were observed in TNF- α and IL-2 levels ($p < 0.05$).

HCV patients

AST levels ranged from 20.2 to 68.8 U/L (mean value 38.9), ALT levels ranged from 27.6 to 136.6 U/L (mean value 64.0), ALP levels ranged from 21.7 to 91.5 U/L (mean value 67.8), GGT levels ranged from 21.7 to 371.6 U/L (mean value 111.9) and total bilirubin levels ranged from 0.54 to 1.53 mg/dL (mean value 0.90). IL-6 levels ranged from 1.447 to 12.029 pg/mL (mean 2.501). Positive correlation was observed between IL-6 levels and ALT ($r=0.436$), as well as between IL-6 levels and total bilirubin ($r=-0.654$) (Fig. 4). TNF- α levels ranged from 2.676 to 15.353 pg/mL (mean 7.519). A trend of positive correlation between TNF- α levels and γ -GT ($r=0.254$) and a

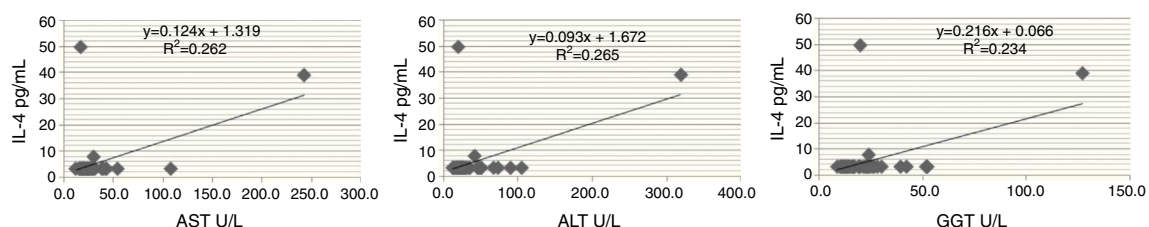


Fig. 3 – Relationship between serum AST, ALT, GGT and circulating IL-4 in HBV patients.

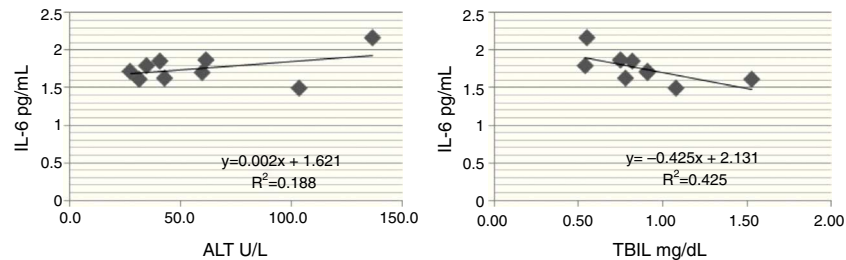


Fig. 4 – Relationship between serum ALT and total bilirubin and circulating IL-6 in HCV.

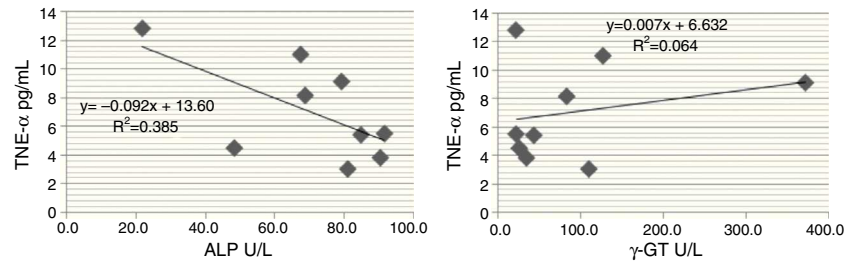


Fig. 5 – Relationship between serum ALP and GGT and circulating TNF-α in HCV patients.

trend of negative correlation between TNF-α levels and total bilirubin levels ($r = -0.250$) were observed too. Significant negative correlation was also observed between TNF-α levels and ALP levels ($r = -0.622$ (Fig. 5). IL-10 levels ranged from 2.851 to 79.614 pg/mL (mean 12.431). IL-10 levels were correlated positively with AST ($r = 0.565$), ALT ($r = 0.777$) and negatively with total bilirubin ($r = -0.404$) (Fig. 6). IL-2 levels ranged from 2.627 to 4.759 pg/mL (mean 3.263). A trend of negative correlation between IL-2 levels and total bilirubin ($r = -0.257$) was observed. Negative correlation was also observed between IL-2 and AST ($r = -0.656$) and ALT ($r = -0.642$) (Fig. 7). IL-4 levels

ranged from 3.209 to 83.705 pg/mL (mean 9.640). No correlation of IL-4 levels with any of the biochemical parameters was observed.

Mean IL-6, and IL-2 levels were different in HCV patients than in healthy controls but these differences were not statistically significant. Statistically significant differences between HCV patients and healthy controls were observed in TNF-α, IL-10 and IL-4 levels ($p < 0.05$).

Statistically significant differences between HBV and HCV patients were observed in IL-2 and IL-10 levels ($p < 0.05$). All study measurements are summarized in Table 1.

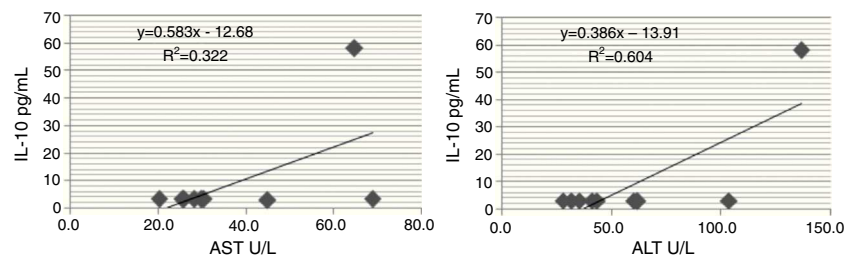


Fig. 6 – Relationship between serum AST and ALT and circulating IL-10 in HCV patients.

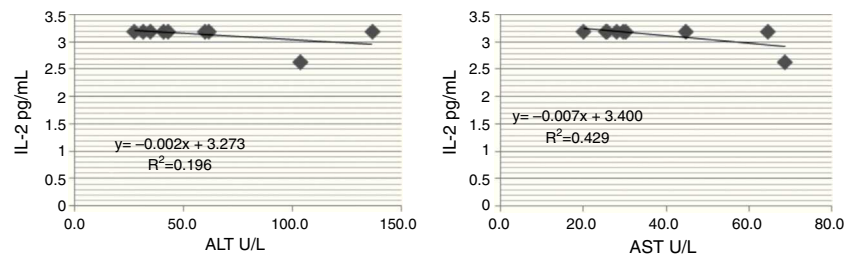


Fig. 7 – Relationship between serum AST and ALT and circulating IL-2 in HCV patients.

Table 1 Summary of all study measurements regarding patients and healthy controls

	Low	High	Mean	Mean standard error
HBV				
AST (U/L)	11.6	242.4	34.0	4.941
ALT (U/L)	11.9	319.1	41.2	6.579
ALP (U/L)	36.4	116.5	64.0	2.686
GGT (U/L)	8.7	127.3	24.9	2.684
Total bilirubin (mg/dL)	0.35	10.77	1.11	0.213
Viral load (U/mL)	14.8	3650000.0		
IL-6 (pg/mL)	1.433	16.123	2.287	0.310
TNF- α (pg/mL)	1.522	46.944	7.112	0.980
IL-10 (pg/mL)	<2.600			
IL-2 (pg/mL)	3.200	136.107	8.542	2.658
IL-4 (pg/mL)	3.200	49.732	5.765	1.163
HCV				
AST (U/L)	20.2	68.8	38.9	7.530
ALT (U/L)	27.6	136.6	64.0	8.762
ALP (U/L)	21.7	91.5	67.8	0.331
GGT (U/L)	21.7	371.6	111.9	7.571
Total bilirubin (mg/dL)	0.54	1.53	0.90	4.756
Viral load (U/mL)	38.0	26300000.0		
IL-6 (pg/mL)	1.447	12.029	2.501	2.504
TNF- α (pg/mL)	2.676	15.353	7.519	0.046
IL-10 (pg/mL)	2.851	79.614	12.431	2.152
IL-2 (pg/mL)	2.627	4.759	3.263	0.277
IL-4 (pg/mL)	3.209	83.705	9.640	0.491
Healthy controls				
IL-6 (pg/mL)			0.744	
TNF- α (pg/mL)			1.323	
IL-10 (pg/mL)			2.130	
IL-2 (pg/mL)			1.668	
IL-4 (pg/mL)			1.765	

Discussion

Chronic hepatitis B and C are characterized by inflammatory liver disease of variable severity and increased risk of developing cirrhosis, liver failure and hepatocellular carcinoma. Hepatitis B virus (HBV) and hepatitis C virus (HCV) are hepatotropic, noncytopathic viruses and liver injury induced by both viruses is mainly mediated by the host immune response against the virus infected cells and by the production of inflammatory cytokines.¹⁸ Changes in various cytokine activities that occur during the inflammatory response against these viruses are responsible for variable degree of liver damage.^{15,19,20} In this study we examined the serum levels of IL-6, TNF- α , IL-10, IL-2 and IL-4 in patients with chronic hepatitis B or C. Moreover, we examined correlations between serum levels of these cytokines with biochemical markers of liver disease and we compared cytokine levels between each patients type and healthy controls, as well as between HBV and HCV patients (Fig. 8).

IL-6 is a pleiotropic cytokine that exerts its complex biological activities through different mechanisms.^{21,22} Elevated levels of IL-6 have been associated with morbidity and disease activity in a variety of chronic diseases^{23,24} as well as in chronic hepatitis, liver cirrhosis and hepatocellular carcinoma.²⁵⁻²⁸ In our study IL-6 levels in HBV patients were significantly different than in healthy controls, in particular 2.287 pg/mL compared to 0.787 pg/mL. IL-6 levels were also

significantly different in HCV patients and in particular 2.501 pg/mL compared to 0.787 pg/mL. However, neither the difference between HBV and HCV patients nor the difference between each type of patients and healthy controls was statistically significant. IL-6 was positively correlated with AST, ALT and GGT in HBV patients. Positive correlation of IL-6 levels with serum ALT levels in HBV patients have also been reported in other studies.^{11,13,29-31} In HCV patients IL-6 was positively correlated with ALT and negatively with total

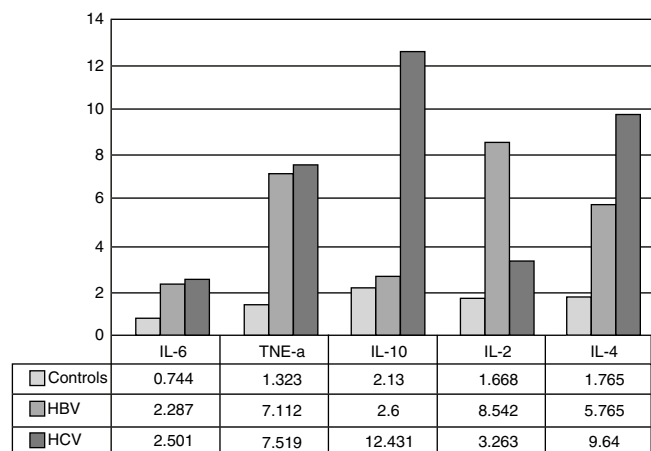


Fig. 8 – Comparison of cytokine levels between patients and controls.

bilirubin. The positive correlation with transaminase levels in both patient groups is suggesting that IL-6 levels are closely related with liver inflammation status.³²⁻³⁴

In liver physiology TNF- α has a dual effect due to its capacity to induce both hepatocyte cell death and hepatocyte proliferation.^{35,36} Circulating TNF- α levels increase during HBV and HCV infection and they are associated with severity of hepatic inflammation, fibrosis, and liver injury. Moreover, they are also elevated in patients with HCC.³⁷⁻⁴¹ In our study, the concentration of TNF- α in the sera of HBV and HCV patients was higher than in the control group, and this difference was statistically significant in both groups ($p < 0.05$). There was no statistically significant difference between the two groups of patients. Serum TNF- α levels were not significantly correlated with serum ALT and AST activities neither in HBV nor in HCV infected patients. Such results have also been reported by other authors and it is concluded that measurement of TNF- α levels reflects liver injury despite normal levels of liver enzymes.^{20,42-46} Elevated serum TNF- α levels have been observed by other researchers even in patients with mild liver inflammation, indicating that this cytokine could be used as a predictor of liver inflammation.⁴⁷

IL-10 plays an anti-inflammatory role in the immune system because it inhibits the production of proinflammatory cytokines and limits T cell activation and differentiation.⁴⁸⁻⁵² Due to its immunoregulatory action, it has been assumed that inadequate levels of IL-10 can determine long-term escape of pathogens from immune control and give rise to persistent infections.^{53,54} In our study IL-10 levels in all HBV patients were very low. Song et al. have reported low IL-10 levels in HBV patients with no association with clinical progression of the infection.⁵⁵ Our finding is in contrast to many studies which have reported elevated IL-10 levels in HBV patients.^{11,13,15} IL-10 levels in HCV patients were statistically different from healthy controls ($p < 0.05$). There are contradictory reports in the literature about IL-10 levels in chronic HCV patients. Some authors have reported decreased levels of IL-10, while others have elevated levels, higher than healthy controls.^{13,15,48-50,56,57} In our study, IL-10 levels were positively correlated with AST and ALT levels and negatively with total bilirubin. Increase of serum AST and ALT concentrations constitutes a reliable marker of necroinflammatory activity. Therefore, we can conclude that the elevated levels of IL-10 in our HCV patients reflect the degree of necroinflammation.^{56,58}

- IL-2 exerts a wide spectrum of effects on the immune system. In our study IL-2 levels in HBV patients were higher than in healthy controls and this difference was statistically significant ($p < 0.05$). In HCV patients, IL-2 levels were different than in healthy controls, in particular 3.263 pg/mL compared to 1.668 pg/mL but this difference was not statistically significant. IL-2 levels were significantly different between HBV and HCV patients ($p < 0.05$). High positive correlation was observed between IL-2 levels and AST, ALT and GGT in HBV patients, suggesting that IL-2 expression correlates with the degree of necroinflammation.^{11,13,59-64} In HCV patients we observed a negative correlation between IL-2 and AST ($r = -0.656$) and between IL-2 and ALT ($r = -0.642$). Our findings agree with previous studies that reported a negative correlation between ALT levels and IL-2 expression

in chronic HCV patients who had persistently normal serum transaminase levels. In these patients the progression of the disease is absent or very slow.^{65,66}

IL-4 has a suppressive effect on HBV gene expression. Thus, it plays an important and complex role in regulating the expression of viral genes and in the innate defense mechanism in liver.⁶⁷⁻⁶⁹ There are contradictory reports about IL-4 levels in chronic hepatitis patients. Some authors have reported lower levels of IL-4 in HCV patients, while others significantly elevated such levels.^{44,45,56,70,71} In our study the concentration of IL-4 in the sera of HBV and HCV patients was different than in the healthy controls. In particular IL-4 mean levels were 5.765 pg/mL in HBV patients and 9.640 pg/mL in HCV patients compared to 1.765 pg/mL in healthy controls, but only in HCV patients this difference was statistically significant ($p < 0.05$). Positive correlation was observed between IL-4 levels and AST, ALT and GGT suggesting that IL-4 expression correlates with the degree of necroinflammation. In HCV patients correlation of IL-4 levels with any of the biochemical parameters was not observed.

Our study revealed a positive significant correlation between IL-6, IL-2 and IL-4 levels on one hand and GGT levels on the other hand in HBV patients. Such a correlation in HCV patients was observed only with TNF- α . Rise of GGT levels indicates hepatocytic dysfunction or obstruction of extra-hepatic bile duct. In HCV patients we also observed such a positive correlation between TNF- α levels and ALP. In conditions affecting the liver, damaged liver cells release increased amounts of ALP into the blood, indicating liver damage. In HBV patients, correlation of total bilirubin levels with any of the cytokines was not observed. Such a correlation was observed only in HCV patients between total bilirubin on one hand and IL-6, TNF- α , IL-10 and IL-2 on the other hand. Bilirubin is a breakdown product of hemoglobin and is usually measured to screen for jaundice caused by liver or gall bladder dysfunction. To our knowledge, such correlations are reported in very few studies. As a general remark we can argue that such correlations reflect the degree of activity of the inflammatory process in the liver, as also stated by other researchers.⁷²⁻⁷⁴

Conclusions

Cytokines play an important role in defending the host against HBV and HCV, but they have also been implicated in the hepatocellular injury seen in the majority of chronically infected patients. Persistent infection modifies the balance between different cytokine, leading to prolonged inflammation, necrosis, fibrosis and chronic liver disease. This study evaluated the serum cytokine levels (IL-6, TNF- α , IL-10, IL-2 and IL-4) of chronic hepatitis B or C patients. HBV patients showed statistically significant difference in TNF- α and IL-2 levels, versus healthy controls. HCV patients showed statistically significant difference in TNF- α , IL-10 and IL-2 levels versus healthy controls. IL10 and IL-2 levels were significantly different between HBV and HCV patients. Correlations of cytokine levels with biochemical markers of liver disease were also observed. Additional studies on a large number of patients may be required to observe the changes of cytokine activities.

Conflict of interest

The authors declare no conflict of interest.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this investigation.

Confidentiality of data. The authors declare that no patient data appears in this article.

Right to privacy and informed consent. The authors declare that no patient data appears in this article.

REFERENCES

- Ott JJ, Stevens GA, Groeger B, Wiersma ST. Global epidemiology of hepatitis B virus infection: new estimates of age-specific HBsAg seroprevalence and endemicity. *Vaccine*. 2012;30:2212-9.
- Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. *J Viral Hepat*. 2004;11:97-107.
- McMahon BJ. Epidemiology and natural history of hepatitis B. *Semin Liver Dis*. 2005;25 Suppl. 1:3-8.
- Averhoff FM, Glass N, Holtzman D. Global burden of hepatitis C: considerations for healthcare providers in the United States. *Clin Infect Dis*. 2012;55(S1):S10-5.
- Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. *Lancet Infect Dis*. 2005;5:558-67.
- Lavanchy D. The global burden of hepatitis C. *Liver Int*. 2009;29 Suppl. 1:74-81.
- Pan CQ, Zhang JX. Natural history and clinical consequences of hepatitis B virus infection. *Int J Med Sci*. 2005;2:36-40.
- Chen SL, Morgan TR. The natural history of hepatitis C (HCV) infection. *Int J Med Sci*. 2006;3:47-52.
- Reherman B, Nascimbeni M. Immunology of hepatitis B virus and hepatitis C virus infection. *Nat Rev Immunol*. 2005;5:215-29.
- Jacobson Brown PM, Neuman MG. Immunopathogenesis of hepatitis C viral infection: Th1/Th2 responses and the role of cytokines. *Clin Biochem*. 2001;34:167-71.
- Falasca K, Ucciferri C, Dalessandro M, Zingariello P, Mancino P, Petrarca C, et al. Cytokine patterns correlate with liver damage in patients with chronic hepatitis B and C. *Ann Clin Lab Sci*. 2006;36:144-50.
- Larrubia JR, Benito-Martínez S, Miquel-Plaza J, Sanz-de-Villalobos E, González-Mateos F, Parra T. Cytokines – their pathogenic and therapeutic role in chronic viral hepatitis. *Rev Esp Enferm Dig*. 2009;101:343-51.
- Khan S, Bhargava A, Pathak N, Maudar KK, Varshney S, Mishra PK. Circulating biomarkers and their possible role in pathogenesis of chronic hepatitis B and C viral infections. *Ind J Clin Biochem*. 2011;26:161-8.
- Prümägi L, Tefanova V, Tallo T, Schmidt E. The role of serum Th1 and Th2 cytokines in patients with chronic hepatitis B and hepatitis C virus infection. *Acta Medica Lituanica*. 2005;12:28-31.
- Mishra PK, Bhargava A, Vashistha P, Varshney S, Maudar KK. Mediators of the immune system and their possible role in pathogenesis of chronic hepatitis B and C viral infections. *AsPac J Mol Biol Biotechnol*. 2010;18:341-9.
- al.Sharif FM. Interleukins and chronic hepatitis. *Middle East J Sci Res*. 2011;7:1001-7.
- Bertoletti A, Gehring AJ. The immune response during hepatitis B virus infection. *J Gen Virol*. 2006;87:1439-49.
- Wieland SF, Chisari FV. Stealth and cunning: hepatitis B and hepatitis C viruses. *J Virol*. 2005;79:9369-80.
- Iannacone M, Sitia G, Guidotti L. Pathogenetic and antiviral immune responses against hepatitis B virus. *Future Virol*. 2006;1:189-96.
- Sobchak DM, Monakova EA. Immune parameters in patients with chronic hepatitis C and with various histologic changes. *Klin Med (Mosk)*. 2004;82:49-52.
- Toumpanakis D, Vassilakkopoulos T. Molecular mechanisms of action of interleukin-6 (IL-6). *Pneumon*. 2007;2:154-67.
- Rose-John S. Interleukin-6 biology is coordinated by membrane bound and soluble receptors. *Acta Biochim Pol*. 2003;50:603-11.
- Gabay C. Interleukin-6 and chronic inflammation. *Arthritis Res Ther*. 2006;8 Suppl. 2:S3.
- Cronstein BN. Interleukin-6 – a key mediator of systemic and local symptoms in rheumatoid arthritis. *Bull NYU Hosp Joint Dis*. 2007;65 Suppl. 1:S11-5.
- Soresi M, Giannitrapani L, D'Antona F, Florena AM, La Spada E, Terranova A, et al. Interleukin-6 and its soluble receptor in patients with liver cirrhosis and hepatocellular carcinoma. *World J Gastroenterol*. 2006;12:2563-8.
- Abd EL-Ghaffar N, Rasheed WI, Ramzy T, El Batae H. Prognostic significance of interleukins determination in liver diseases research. *J Med Med Sci*. 2008;3:124-31.
- Wong VW, Yu J, Cheng AS, Wong GL, Chan HY, Chu ES, et al. High serum interleukin-6 level predicts future hepatocellular carcinoma development in patients with chronic hepatitis B. *Int J Cancer*. 2009;124:2766-70.
- Kuo T-M, Hu C-p, Chen Y-L, Hong M-H, Jeng K-S, Liang C-CT, et al. HBV replication is significantly reduced by IL-6. *J Biomed Sci*. 2009;16:41.
- Chen J, Wang Y, Wu X-J, Li J, Hou F-Q, Wang G-Q. Pegylated interferon α -2b up-regulates specific CD8+ T cells in patients with chronic hepatitis B. *World J Gastroenterol*. 2010;16:6145-50.
- Lokhande MU, Miquel J, Benito S, Larrubia J-R. HBV & HCV Immunopathogenesis, Viral Hepatitis – Selected Issues of Pathogenesis and Diagnostics, Dr. Sergey Mukomolov (Ed.), ISBN: 978-953-307-760-4, InTech. Available from: <http://www.intechopen.com/books/viralhepatitis-selected-issues-of-pathogenesis-and-diagnostics/hbv-hcv-immunopathogenesis>
- Gao B. Cytokines, STATs and liver disease. *Cell Mol Immunol*. 2005;2:92-100.
- Zhang L, Miao L, Han F, Dou XG. Cytokine levels in serum of patients with chronic hepatitis C and its significance. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi*. 2011;27:301-3.
- Nakagawa H., Maeda S., Yoshida H., Tateishi R., Masuzaki R., Ohki T., et al. Serum IL-6 levels and the risk for hepatocarcinogenesis in chronic hepatitis C patients: an analysis based on gender differences. *Int J Cancer*. 2009;125:2264-9.
- Tangkijvanich P, Vimolket T, Theamboonlers A, Kullavanijaya P, Suwangool P, Poovorawan Y. Serum interleukin-6 and interferon-gamma levels in patients with hepatitis B-associated chronic liver disease. *Asian Pac J Allergy Immunol*. 2000;18:109-14.
- Schwabe RF, Brenner DA. Mechanisms of liver injury: TNF- α -induced liver injury: role of IKK, JNK, ROS pathways. *Am J Physiol Gastrointest Liver Physiol*. 2006;290:G583-9.

36. Wullaert A, van Loo G, Heynink K, Beyaert R. Hepatic TNF signaling and NF- κ B: effects on liver homeostasis and beyond. <http://edrv.endojournals.org/content/early/2007/04/12/er.2006-0031.full.pdf>
37. Akpolat N, Yahsi S, Godekmerdan A, Demirbag K, Yalniz M. Relationship between serum cytokine levels and histopathological changes of liver in patients with hepatitis B. *World J Gastroenterol.* 2005;11:3260-3.
38. Yia Y-S, Parka SG, Byeona SM, Kwonb Y-G, Jung G. Hepatitis B virus X protein induces TNF- α expression via down-regulation of selenoprotein P in human hepatoma cell line, HepG2. *Biochim Biophys Acta.* 2003;1638: 249-56.
39. Liu G, Tang K, Li Q, Yuan G, Cao W, Lu W. Changes of IL-1, TNF- α , IL-12 and IL-10 levels with chronic liver failure. *Surg Sci.* 2011;2:69-72.
40. Thio CL, Goedert JJ, Mosbrugger T, Vlahov D, Strathdee SA, O'Brien SJ, et al. An analysis of tumor necrosis factor a gene polymorphisms and haplotypes with natural clearance of hepatitis C virus infection. *Genes Immun.* 2004;5: 294-300.
41. Mogensen TH, Paludan SR. Molecular pathways in virus-induced cytokine production. *Microbiol Mol Biol Rev.* 2001;65:131-50.
42. Jeng J-E, Tsaiz J-F, Chuang L-Y, Ho M-S, Linz Z-Y, Hsieh M-Y, et al. Tumor necrosis factor- α 308.2 polymorphism is associated with advanced hepatic fibrosis and higher risk for hepatocellular carcinoma. *Neoplasia.* 2007;9:987-92.
43. Wang YY, Lo GH, Lai KH, Cheng JS, Lin CK, Hsu PI. Increased serum concentrations of tumor necrosis factor- α are associated with disease progression and malnutrition in hepatocellular carcinoma. *J Chin Med Assoc.* 2003;66: 593-8.
44. Astakhin AV, Levitan BN, Afanas'ev SS, Vorob'ev AA, Aleshkin VA, Dudina OS. Tumor necrosis factor- α and interleukin-4 in the blood sera of chronic hepatitis patients. *Zh Mikrobiol Epidemiol Immunobiol.* 2004;46-50.
45. Loginov AS, Tsaregorodtseva TM, Zotina MM, Serova TI, Iakimchuk GN, Fedotova TF. Interleukins in chronic viral hepatitis. *Ter Arkh.* 2001;73:17-20.
46. Helaly GF, Abou Shamaa LA. Tumor necrosis factor- α in hepatitis C virus infection: a correlation with viral activity and serum aminotransferases. Available at: <http://ejimmunology.tripod.com/previous/jun02/jun02-10.htm>
47. Moura AS, Carmo RA, Teixeira AL, da Costa Rocha MO. Soluble inflammatory markers as predictors of hepatocellular damage and therapeutic response in chronic hepatitis C. *Braz J Infect Dis.* 2009;13:375-82.
48. Zdanov A. Structural features of the interleukin-10 family of cytokines. *Curr Pharm Des.* 2004;10:3873-84.
49. Moore KW, de Waal MR, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. *Annu Rev Immunol.* 2001;19:683-765.
50. Asadullah K, Sterry W, Volk HD. Interleukin-10 therapy-review of a new approach. *Pharmacol Rev.* 2003;55:241-69.
51. Boyer N, Marcellin P. Pathogenesis, diagnosis and management of hepatitis C. *J Hepatol.* 2000;32: 98-112.
52. Pestka S, Krause CD, Sarkar D, Walter MR, Shi Y, Fisher PB. Interleukin-10 and related cytokines and receptors. *Annu Rev Immunol.* 2004;22:929-79.
53. Bruno CM, Valenti M, Bertino G, Ardiri A, Amoroso A, Consolo M, et al. Relationship between circulating interleukin-10 and histological features in patients with chronic C hepatitis. *Ann Saudi Med.* 2011;31:360-4.
54. Kamal S, Ismail M, Shaker H, Moustafa H. Serum and intrahepatic cytokine patterns in hepatitis B and C. *Egypt J Med Lab Sci (ESIC).* 2003;12.
55. Song le H, Binh VQ, Duy DN, Kun JF, Bock TC, Kremsner PG, et al. Serum cytokine profiles associated with clinical presentation in Vietnamese infected with hepatitis B virus. *J Clin Virol.* 2003;28:93-103.
56. Gao Q-J, Liu D-W, Zhang S-Y, Jia M, Wang L-M, Wu L-H, et al. Polymorphisms of some cytokines and chronic hepatitis B and C virus infection. *World J Gastroenterol.* 2009;15: 5610-9.
57. Karim F, Rahman S, Khan M, Alam AK, Ahmed N, Zaki K, et al. Serum Interleukin-10 level in patients with chronic hepatitis B infection. *Hungar Med J.* 2007;1:241-50.
58. McCaughan GW, George J. Fibrosis progression in chronic hepatitis C virus infection. *Gut.* 2004;53:318-21.
59. Abayli B, Canataroğlu 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.
60. Sofian M, Aghakhani A, Farazi AA, Banifazl M, Eslamifar A, Rashidi N, et al. Serum profile of T helper 1 and T helper 2 cytokines in hepatitis C virus infected patients. *Hepat Mon.* 2012;12:6156.
61. Malek TR, Castro I. Interleukin-2 receptor signaling: at the interface between tolerance and immunity. *Immunity.* 2010;33:153-65.
62. Bachmann MF, Oxenius A. Interleukin 2: from immunostimulation to immunoregulation and back again. *EMBO Rep.* 2007;8:1142-8.
63. Xiao P, Chen Q-F, Yang Y-L, Guo Z-H, Chen H. Serum soluble interleukin-2 receptor levels in patients with chronic hepatitis B virus infection and its relation with anti-HBc. *World J Gastroenterol.* 2006;12:482-4.
64. Spanakis NE, Garinis GA, Alexopoulos EC, Patrinos GP, Menounos PG, Sklavounou A, et al. Cytokine serum levels in patients with chronic HCV infection. *J Clin Lab Anal.* 2002;16:40-6.
65. Gramenzi A, Andreone P, Loggi E, Foschi FG, Cursaro C, Margotti M, et al. Cytokine profile of peripheral blood mononuclear cells from patients with different outcomes of hepatitis C virus infection. *J Viral Hepat.* 2005;12: 525-30.
66. 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;118:760-4.
67. Zamorano J, Rivas MD, Pérez M. Interleukin-4: a multifunctional cytokine. *Inmunología.* 2003;22:215-24.
68. Aoudjehane L, Podevin P, Scatton O, Jaffray P, Dusanter-Fourt I, Feldmann G, et al. Interleukin-4 induces human hepatocyte apoptosis through a Fas-independent pathway. *FASEB J.* 2007;12:1433-44.
69. Lin S-J, Shu P-Y, Chang C, Ng A-K, Hu C-p. IL-4 suppresses the expression and the replication of hepatitis B virus in the hepatocellular carcinoma cell line Hep3B. *J Immunol.* 2003;171:4708-16.
70. Gigi E, Raptopoulou-Gigi M, Kalogeridis A, Masiou S, Orphanou E, Vrettou E, et al. Cytokine mRNA expression in hepatitis C virus infection: TH1 predominance in patients with chronic hepatitis C and TH1-TH2 cytokine profile in subjects with self-limited disease. *J Viral Hepat.* 2008;15:145-54.
71. Nejatollahi F, Davarpanah MA, Hosseini SH. Interferon- γ , Interleukin-10 and interleukin-4 in the blood sera of patients with chronic hepatitis B virus infection in Shiraz. In: 4th international Tehran hepatitis congress. 2011.
72. Reuter S, Schneckeburger M, Cristofanon S, Buck I, Teiten MH, Daubeuf S, et al. Tumor necrosis factor α induces g-glutamyltransferase expression via nuclear factor- κ B in

- cooperation with Sp1. *Biochem Pharmacol.* 2009;77:397-411.
73. Ding Y, Zhao L, Mei H, Huang Z-H, Zhang S-L. Alterations of biliary biochemical constituents and cytokines in infantile hepatitis syndrome. *World J Gastroenterol.* 2006;12:7038-41.
74. Farghaly ME, El-Din Bekhit MN, Fahmy K, Gharieb AF. Impact of serum interleukin-10 in pathogenesis of chronicity in hepatitis C virus patients. *Egypt J Med Microbiol.* 2007;16:301-10.