

# Use of Serum Levels of Proinflammatory Cytokine IL-1 $\alpha$ in Chronic Hepatitis C

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## ABSTRACT

*Immunoregulatory cytokines influence the persistence of hepatitis C virus chronic infection and the extent of liver damage. Interleukin-1 plays an important role in the inflammatory process. Some studies have demonstrated that IL-1 production was impaired in patients with chronic infections of hepatitis C virus, implying that IL-1 may play a role in viral clearance. In this study, along with routine laboratory tests, has been performed the analysis of serum levels of proinflammatory cytokine IL-1 $\alpha$  in order of better understanding and monitoring of chronic hepatitis C. The aim of study was to analyze the usefulness of laboratory tests, which are routinely used in the assessment of liver disease with specified immunological parameters, in patients with chronic hepatitis C. Total of 60 subjects were divided into two groups: HCV-PCR positive and negative group. The control group of 30 healthy participants was included. Apart from standard laboratory tests, the analysis included serum levels of cytokine IL-1 $\alpha$ . IL-1 $\alpha$  had the highest mean concentration in group of viral hepatitis C, with PCR positive test (5.73 pg / mL), and then in of chronic viral hepatitis C, PCR negative test (5.39 pg / mL). ANOVA test proves that IL-1 $\alpha$  in the healthy group was different from other groups as follows: in relation to HCV- RNA-PCR positive patients statistical significance level was  $p < 0.001$  ( $F=32\ 755$ ); in relation to HCV- RNA-PCR negative was also statistically significant at  $p < 0.001$  ( $F=182\ 361$ ); Cytokine IL-1 was statistically analyzed separately and compared by group 1 and 2 using Student *t*-test for independent samples. Statistical significance was observed at  $p=0.026$ . IL-1 $\alpha$  was positively correlated with the duration of the illness ( $p < 0.01$ ) and with serum ALT activity ( $p < 0.01$ ) and serum AST activity ( $p < 0.01$ ). Using multivariate analysis model »Factor Analysis«, was made significant stratification predictive parameters in relation to the cytokine IL-1 $\alpha$ , stratified significance is indicated as follows: 1. Age, 2. history of receiving transfusions, 3. ALT, 4. AST, 5. MELD score (negative), 6. Child-Pugh score (negative). IL-1 $\alpha$  was significantly elevated in inflammatory conditions of pronounced activity (PCR positive hepatitis). IL-1 $\alpha$  may have important role as marker of both inflammation and hepatic injury, particularly in the course of hepatitis C. Results suggest that inflammatory and immune parameters, analyzed together can significantly contribute to the understanding and predicting of chronic liver damage.*

**Key words:** chronic hepatitis C, parameters of inflammation, IL- 1 $\alpha$ .

## Introduction

Primary HCV infection is asymptomatic in the majority of cases, with 50–80% of individuals developing chronic infection<sup>1,2</sup>. The immune response is a key component in the activation and maintenance of antiviral immunity, through induction of cytokines and initiation of the adaptive immune response. Clearance of chronic hepatitis C virus (HCV) has been associated with early, multi-specific and sustained CD4<sup>+</sup> and CD8<sup>+</sup> responses directed against HCV epitopes<sup>3–12</sup>. The pro-inflammatory cytokines are involved in viral clearance and in metabolic and viral hepatic diseases. Th1 cytokines positively correlate with hepatic inflammation in HCV infection<sup>13–17</sup>. Func-

tional impairment, suppression or deletion of antigen-specific T cells appears to be a key determinant of progression to chronicity<sup>11,18</sup>. The importance of the cytokine milieu in determining viral clearance has been emphasized by recent studies<sup>19–21</sup>. Cytokines are low-molecular-weight mediators of cellular communication produced by multiple cell types in the liver, with the Kupffer cell critically important. Proinflammatory cytokines such as interleukin-1, tumor necrosis factor and interleukin-8 are acute-phase cytokines and play a role in the liver injury of acute and chronic liver diseases<sup>22</sup>.

Interleukin-1 (IL-1) has central role in inflammatory process especially acute inflammation. It is an indicator of the intensity of inflammation activity<sup>23</sup>. Some studies have demonstrated that IL-1 production was impaired in patients with chronic hepatitis C, implying that IL-1 may play a role in viral clearance<sup>19–23</sup>.

The objective of this study was to determine and analyze serum level of IL-1 $\alpha$  in patients with chronic hepatitis C in correlation of the presence of viral genetic replication and functional liver status.

## Patients and Methods

The study was conducted as one year, an open, case controlled comparative clinical trial.

Before entering the study, each patient reviewed and signed an informed consent. All research described in study, involving human subjects and eterial derived from human subjects complied with ethnical principles. Standards of Good Clinical Practice, Good Laboratory Practice and The declaration of Helsinki were followed.

The study was conducted at the Department of gastroenterology and Hepatology, Clinical Centre University of Sarajevo.

### Patients

Total of 90 patients of either sex, 18–80 years of age, were recruited.

Inclusion criteria were patients with positive serum HCV antibodies, polymerase chain reaction test was performed. Previous liver biopsy diagnosis of hepatitis C was needed, no more than 6 months before study. Duration of illness was measured according to first notification of liver damage and Anti HCV positivity. Exclusion criteria were: presence of liver disease caused by NAFLD/NASH, hereditary condition, cardiac liver cirrhosis, liver disease occurred during pregnancy, vascular disease of the liver, primary biliary cirrhosis, as and subjects with liver transplant, acute hepatitis, evidence of acute or chronic inflammatory syndrome of other known origin, immunodeficiency states.

Respondents with diagnosis od chronic hepatitis C – 60 patients were divided in two groups:

1. Chronic hepatitis C, HCV-RNA-PCR positive test, anti HCV positive
2. Chronic hepatitis C, HCV-RNA-PCR negative test, anti HCV positive.

A control group of 30 healthy subjects was included as well as group 3.

### Methods

A physical examination was carried out and medical history was taken during the pre-study visit. The following data were recorded from all patients: age, gender,

BMI, history of narcotics consumption or transfusion receiving, history of liver disease – liver biopsy, comorbidity.

Biochemical parameters were recorded: full blood count, international normalized ratio (INR), active partial thromboplastin time (APTT) and routine liver function tests including bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase and gamma-glutamyl transferase, proteinogram. Polymerase chain reaction for detecting viral RNA in serum was used.

Serum levels of cytokine IL-1 $\alpha$  where determined and measured by quantative Sandwich Enzyme Immunoassay technique. Monoclonal antibodies specific for IL-1 $\alpha$  were coated on mikroparticules, which come into contact with the sample, and is based on the occurrence of a specific color, measuring the intensity, extent and concentration of cytokine in the sample.

Analysis was made from frozen serum samples that were collected in serum separator tube. Values were expressed as pg/ml.

Functional status of the liver was determined by the modified Child Pugh and MELD scores.

### Statistical analysis

For the processing of data computer program »Statistica for Windows 5.0« was used. Categorical data were expressed as proportions (%), and continuous data as means ( $\bar{X}$ )  $\pm$  standard deviation (SD). Statistical methods used in this study where: analysis of variance test (ANOVA test), multivariate analysis model »Factor Analysis«, and Student's t-test for independent samples. The level of significance was  $p < 0.05$ .

## Results

A total of 90 subjects, divided into two groups of 30 patients, also with a group of 30 healthy subjects treated for comparison. The Table 1 shows the basic demographic and anthropological parameters of subjects per group, which were not significantly different by group ( $p > 0.05$ ).

**TABLE 1**  
BASIC DEMOGRAPHIC AND ANTHROPOLOGICAL  
PARAMETERS OF SUBJECTS PER GROUP

Group	Age (years) (SD)	TT (kg) (SD)	TV (cm) (SD)	BMI (SD)
1	44.47 (9.82)	81.00 (11.82)	171.00 (8.10)	23.29 (2.29)
2	51.93 (13.13)	69.27 (9.23)	171.08 (6.31)	24.48 (2.45)
3	45.91 (8.37)	77.93 (9.34)	175.07 (5.06)	26.94 (2.43)
	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$

Increased enzyme activity was noted in groups of patients with liver disease compared to the control group of healthy subjects ( $p < 0.01$ ). INR and APTT did not significantly differ by groups (Table 2) ( $p > 0.05$ ). The values of total protein were not significantly different (Table 3). There was no statistically significant differences in the analysis of hematological parameters (Table 4).

Figure 1 presents average values of interleukin 1 (IL-1 $\alpha$ ) in groups.

ANOVA analysis of variance test proves that IL-1 $\alpha$  in the healthy group (3) was different from other groups as follows: in relation to group 1 statistical significance level was  $p < 0.001$  ( $F = 32.885$ ); in relation to group 2 was also statistically significant at  $p < 0.001$  ( $F = 180.361$ );

IL-1 $\alpha$  had the highest mean concentration in group 1 – viral hepatitis C, with PCR positive test (5.88 pg/ml), and then in group 2- anti HCV positive, PCR negative test (5.37 pg/ml).

Cytokine IL-1 $\alpha$  was statistically analyzed separately and compared by groups using Student t-test for independent samples. Statistical significance was observed between groups 1 and 2 at  $p = 0.026$ . IL-1  $\alpha$  was positively correlated with the duration of the illness ( $p < 0.01$ ) and with serum ALT activity ( $p < 0.01$ ) and serum AST activity ( $p < 0.01$ ). Using multivariate analysis model »Fac-

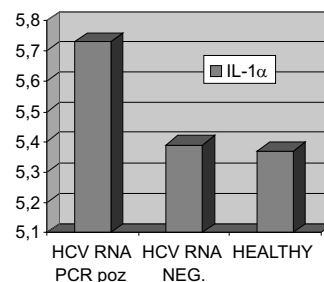


Fig. 1. The average values of interleukin 1  $\alpha$  (pg/ml) in groups. Group 1 – Anti HCV positive, HCV RNA PCR positive. Group 2 – Anti HCV negative, HCV RNA PCR negative. Group 3 – Healthy subjects. ANOVA  $p < 0.01$ .

tor Analysis», was made significant stratification predictive parameters in relation to the cytokine IL-1 $\alpha$ , stratified significance is indicated as follows: 1. Age, 2. history of receiving transfusions, 3. ALT, 4. AST, 5. MELD score (negative), 6. Child-Pugh score (negative).

## Discussion

In clinical practice, for determining the degree of functional status of liver Child Turcotte Pugh and MELD scoring systems are used. In this study, along with routine lab-

**TABLE 2**  
AVERAGE ACTIVITY VALUES OF ENZYMES, BILIRUBIN, INR AND APTT IN GROUPS

Group	Ala AT IU/mL (SD)	Asp AT IU/mL (SD)	$\gamma$ GT IU/mL (SD)	AP IU/mL (SD)	INR (SD)	APTT (s)	Bil. tot. ( $\mu$ mol/L)	Bil. dir. ( $\mu$ mol/L)
1	77.77 (43.83)	66.30 (44.40)	110.40 (91.31)	87.41 (27.41)	1.23 (0.21)	40.70 (4.51)	21.41 (26.66)	10.72 (9.08)
2	79.91 (81.96)	80.80 (73.76)	70.53 (29.33)	88.50 (50.47)	1.23 (0.20)	37.69 (4.35)	25.56 (18.11)	9.61 (8.16)
3	33.63 (7.24)	25.03 (6.28)	38.70 (7.43)	86.83 (21.17)	1.09 (0.14)	38.82 (3.68)	10.75 (4.31)	7.28 (1.27)
	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p < 0.05$	$p < 0.05$

**TABLE 3**  
STATISTICAL ANALYSIS OF PROTEIN PARAMETERS IN GROUPS.

Group	Tot. proteins (g/L) (SD)	Albumin (g/L) (SD)	Globulin (g/L) (SD)	A/G index (SD)	Fibrinogen (g/L) (SD)
1	75.03 (7.66)	36.70 (5.82)	38.33 (4.93)	1.05 (0.32)	2.56 (0.77)
2	76.80 (6.70)	39.23 (5.11)	36.57 (5.55)	1.11 (0.24)	2.99 (0.76)
3	73.47 (5.81)	40.63 (4.26)	32.98 (3.36)	1.25 (0.16)	2.67 (0.78)
	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$

**TABLE 4**  
STATISTICAL ANALYSIS OF HEMATOLOGICAL PARAMETERS IN GROUPS.

Group	Hb (g/L) (SD)	Htc	MCV (fL) (SD)	Le ( $\times 10^9/L$ ) (SD)	Tr ( $\times 10^9/L$ ) (SD)
1	145.10 (15.20)	46.01 (3.89)	95.33 (3.42)	6.14 (1.56)	171.38 (68.50)
2	148.50 (20.56)	42.22 (5.60)	94.87 (8.41)	5.79 (1.81)	168.71 (56.61)
3	143.83 (7.60)	40.33 (3.02)	94.23 (4.84)	6.57 (1.74)	236.13 (60.70)
	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$

oratory tests, has been performed the analysis of serum levels of proinflammatory cytokine IL-1 $\alpha$  in order of better understanding and monitoring of chronic hepatitis C.

Measurements of average values of enzyme activity (AST, ALT,  $\gamma$ GT and AP) and bilirubin in groups showed increased activity of these enzymes in groups of patients with chronic hepatitis compared to controls, which as expected due to chronic liver process. The values of total protein and hematological parameters were not significantly different by groups. Functional indicators of liver damage (Child Pugh and MELD scores) showed a correlation in both groups, but not as good assessment system the intensity of inflammation and involvement of the liver fibrosis process. What explains their primary purpose in evaluating the terminal stages of liver damage.

Statistical analysis of the cytokines analyzed showed very impressive results. An analysis of serum level of Interleukin-1 $\alpha$  showed a high degree of correlation with active replication of genetic material (HCV RNA PCR positive – group 1), resulting in a high degree of statistical significance. The most active inflammatory process occurred in this group, while in group 2 -HCV RNA PCR negative inflammatory reaction has subsided, and has been better controlled, but still present, which is consistent with the results.

The increase of IL-1 $\alpha$  divert an inflammatory reaction of the predominantly exudative – cellular responses, under the influence of IL-1 $\alpha$ , to fibroblast – granulation response. Histologically speaking, at this stage to expect significant activity of fibroblasts, fibrous components of

reproduction in inflammatory region. If this process is extremely intense generated significant predisposition to replace functional liver tissue with fibrosis, which may have long-term pathological changes in liver structure, and then the functional repercussions. At this stage it is necessary to evaluate how favorable is »repair inflammatory reaction«. If it is too intense, the stabilization of the formed fibrous tissue, in the long term, could functionally suppress liver tissue. It follows that monitoring the changes in concentrations of IL-1 and other cytokines (TGF- $\beta$ 1) might have a use value »predictor of cirrhosis«. Studies suggest that elevated levels of IL-1 in samples of liver tissue indicate the pronounced activity of hepatitis C<sup>24</sup> and it is to expect increased serum levels of this cytokine in this conditions, which results from this research.

Recent studies associates levels of cytokines, as immune response parameters, with the expected response to antiviral therapy<sup>25</sup>. IL 1 could here, possibly, find its application, which is the subject of further research.

## Conclusion

Analysis of serum levels of proinflammatory cytokine IL-1 $\alpha$  and functional status of the liver revealed detailed information about the chronic hepatitis. IL-1 $\alpha$  was significantly elevated in inflammatory conditions of pronounced activity (PCR positive hepatitis) and can be used as important parameter of inflammatory activity and fibrosis evaluation in chronic liver damage.

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## KORISNOST RAZINE SERUMA PREDUPALNOG CITOKINA IL-1 $\alpha$ KOD KRONIČNOG HEPATITISA C

### SAŽETAK

Imunoregulatorni citokini utječu na prisutnost kronične infekcije virusom hepatitisa C i na opseg oštećenja jetre. Interleukin-1 igra važnu ulogu u upalnom procesu. Neke studije su pokazale da je IL-1 produkcija umanjena u pacijenata s kroničnom infekcijom s virusom hepatitisa C, što ukazuje da IL-1 ima ulogu u virusnom čišćenju. U ovoj studiji, zajedno s rutinskim laboratorijskim testovima, izvršena je analiza serumske razine proupalnih citokina IL-1 $\alpha$  u cilju boljeg razumijevanja i praćenja kroničnog hepatitisa C. Cilj istraživanja bio je analizirati korisnost laboratorijskih testova koji se rutinski koriste u procjeni bolesti jetre s određenim imunološkim parametrima kod bolesnika s kroničnim hepatitisom C. Ukupno 60 ispitanika bilo je podijeljeno u dvije skupine: HCV PCR pozitivne i negativne grupe. Kontrolna skupina od 30 zdravih učesnika je bila uključena. Osim standardnih laboratorijskih ispitivanja, analizirane su serumske razine citokina IL-1 $\alpha$ . IL-1 $\alpha$  ima najveću srednju koncentraciju u skupini virusnog hepatitisa C, s PCR pozitivnim testom (5,73  $\mu$ g/mL), a zatim kod kroničnog virusnog hepatitisa C, s PCR negativnim testom (5,39  $\mu$ g/mL). ANOVA test pokazuje da je IL-1 $\alpha$  u zdravoj skupini bio je drugačiji od ostalih skupina kako slijedi: u odnosu na HCV RNA PCR pozitivne pacijente statistička granica značajnosti je  $p < 0,001$  ( $F=32\ 755$ ); u odnosu na HCV RNA PCR negativ je također statistički značajno uz  $p < 0,001$  ( $F=182\ 361$ ); citokin IL-1 statistički je analizirano pojedinačno i odnosu prema grupi 1 i 2 pomoću studentovog t-testa za nezavisne uzorke. Uočena je statistička značajnost od  $p=0,026$ . IL-1 $\alpha$  pozitivno je povezana s trajanjem bolesti ( $p < 0,01$ ), serumskim ALT ( $p < 0,01$ ) i aktivnosti seruma AST ( $p < 0,01$ ). Korištenjem multivarijatne analize modela faktorske analize, stratifikacijski prediktivni parametar je značajan u odnosu na citokin IL-1 $\alpha$ , stratificirani značaj prikazan na sljedeći način: 1. dob, 2. povijest primanja transfuzije, 3. ALT, 4. AST, 5. MELD rezultat (negativan), 6. Child-Pugh rezultat (negativan). IL-1 $\alpha$  je značajno povišen u izrazito aktivnim upalnim uvjetima (PCR pozitivan hepatitis). IL-1 $\alpha$  može imati važnu ulogu kao znak i upale i oštećenja jetre, osobito u tijeku hepatitisa C. Rezultati ukazuju na to da analiza upalnih i imunoloških parametara može značajno pridonijeti razumijevanju i predviđanju kroničnog oštećenja jetre.