

Serum Levels of IL-1 Beta and IL-6 In HCV Infected Patients As Markers for Liver Disease Progression

Nagwa Abd EL-Ghaffar*, Wafaa I.E. Rasheed**, Hassan El Batae***.

*The Departments of Clinical and Chemical pathology, ** Medical Biochemistry, National Research Centre, Cairo, Egypt and***Tropical Medicine, Faculty of Medicine, Tanta university.

ABSTRACT

Aim: In this study, we aimed to characterize serum cytokine levels of interleukin-1 Beta (**IL-1 β**) and interleukin -6 (**IL-6**) in HCV infected patients & in patients with hepatocellular carcinoma (HCC) in comparison to control group and their possible use as markers of disease progression.

Patients and Methods: Sixty Patients were divided into three groups: **Group I:** included 20 HCV infected patients without cirrhotic changes. **Group II:** included 20 HCV infected patients with liver cirrhosis (LC). **Group III:** included 20 HCV infected patients with HCC and 20 apparently healthy subjects as control group. All patients and control group were subjected to biochemical and serological tests, anti HCV, HCV (RT-PCR) and cytokines measurements of serum IL-1 β & serum IL-6 levels.

Results: Showed a high statistically significant elevated serum IL-6 and IL-1 β levels in patients with chronic HCV infection in comparison to control group. Highly statistically elevated levels of IL-6 and IL-1 β in liver cirrhosis and higher levels were found in HCC group in comparison to control group. The levels of IL-6 and IL-1 β increased significantly in HCV infected patients as the disease progress.

Conclusion: Serum IL-1 β , and IL-6 levels are elevated in patients with hepatitis C-related liver diseases, especially in LC and HCC patients. Their levels reflect hepatic dysfunction better than liver inflammation parameters; accordingly, we may use serum IL-1 β and IL-6 as markers for Liver disease progression in HCV-infected patients instead of invasive techniques.

Key words: IL-1 beta, IL-6, HCV, and HCC.

INTRODUCTION

Hepatitis C virus (HCV) infection is a worldwide public health problem ⁽¹⁾. HCV infection frequently leads to chronic hepatitis, which may progress to cirrhosis and even to hepatocellular carcinoma (HCC). The mechanisms responsible for the pathogenesis of chronic HCV infection are not well known ⁽²⁾.

Cytokines constitute a complex network of molecules involved in physiologic and pathologic processes going on in the liver, such as liver growth and regeneration, inflammatory

processes including viral liver disease, liver fibrosis and cirrhosis ⁽³⁾.

An increase in serum levels of pro-inflammatory cytokines has been noted in liver diseases, including chronic hepatitis C ⁽⁴⁾. The most important pro-inflammatory cytokines, IL-1 (alpha and beta) are expressed in the liver mostly by resident macrophages (Kupffer cells) and T-cells. Interleukin-1 β gives rise to the cascade of the inflammatory response and recent reports have shown that its levels are higher in HCV-related liver diseases than in

other forms of liver damage ⁽¹⁾. Its polymorphisms behind are related to the risk of progression to HCC ⁽⁵⁾.

Interleukin-6 (IL-6) is a proinflammatory cytokine, which plays an important role in the host defence mechanism. Serum IL-6 levels are low in physiological conditions, but increase considerably in pathological conditions such as trauma, inflammation and neoplasia. In tumours, IL-6 may be involved in promoting the differentiation and growth of target cells ⁽⁶⁾. It has also been hypothesized that activation of the IL-6 gene is responsible for the derangement of some events, which can lead to neoplastic degeneration ⁽⁷⁾.

In this study, we aimed to characterize serum cytokine levels of IL-1 β and IL-6 in HCV infected patients and in HCC patients in comparison to control group and their possible use as markers of disease progression.

MATERIALS AND METHODS

The study was performed in 80 subjects from out and in patients of Tropical Medicine Department, Tanta university hospitals during the period from January 2005 until January 2006.

The present study included 60 patients with chronic hepatitis C who had undergone liver biopsy. They consisted of 40 men and 20 women with ages ranging from 36 to 66 years. The diagnosis of chronic hepatitis C was made on the basis of positivity for anti-HCV (by the second generation ELISA), and confirmed by HCV-RNA reverse transcription-polymerase chain reaction (RT-PCR).

Patients with HBV infection or auto antibodies (antinuclear antibody, anti-smooth muscle antibody, and antimitochondrial antibody), or history of alcohol abuse were excluded from the study.

Patients were divided into three groups:

Group I: included 20 HCV infected patients without cirrhotic changes. It included 11 males, 9 females, with ages ranging from 43 to 66 years. Diagnosis was made by persistently normal alanine aminotransferase (ALT) values for 6 months and no detectable liver changes by sonography except for a bright fatty liver, with

minimal changes in liver biopsy.

Group II: included 20 HCV infected patients with liver cirrhosis, 13 males, 7 females, with ages ranging from 36-62 years. Diagnosis was based on biopsy findings.

Group III: included 20 HCV infected patients with HCC, 16 males, 4 females, with ages ranging from 43-61 years. Diagnosis was based on biopsy and cytological findings.

Twenty subjects with age (44-61 years) and sex (10 males and 10 females) matching characters with no history of liver disease with normal liver enzymes and free ultrasonographic finding were included in this study as control group.

A detailed history and physical examination of the patients were carried out with special emphasis on history of schistosomiasis, prior parenteral therapy, infective hepatitis and jaundice or other signs of liver cell failure. Complete clinical examination, which includes the manifestations of hepatitis and liver cell failure such as jaundice, hepatomegaly, tenderness in the right hypochondrium, ascites, splenomegaly, lower limb oedema as well as abdominal ultrasonography was also done side by side with routine laboratory investigations.

Biochemical and serological tests

Ten millilitres of fasted venous blood (6 Hours of fasting) were taken from each subject participating in the study, and divided into aliquots: The 1st aliquot was taken on EDTA for determination of blood picture. The 2nd aliquot was left to clot and the serum were separated by centrifugation and stored at -20°C for analysis of:

Liver function test: Total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein, albumin and prothrombin time (PT).

Markers of hepatitis virus: Hepatitis B surface antigen (HBsAg), hepatitis B core antibody (HbcAb), hepatitis C virus antibody (HCV-Ab), and hepatitis C virus RNA (HCV-RNA).

Kidney function tests: Serum urea and creatinine.

Blood picture: Haemoglobin (Hb), white blood cell counts (WBCs), red blood cell counts (RBCs) and platelets.

Cytokines measurements: Serum IL-1 β & IL-6.

Liver function tests: Liver function tests were performed using a Beckman Auto-analyzer (Synchron CX4, USA). A diazotization method was used for determination of serum total bilirubin based on Malloy and Evelyn, 1937⁽⁸⁾. Activities of ALT and AST were measured by the enzyme rate method⁽⁹⁾; Albumin was determined according to Pinnell and Northam, 1978⁽¹⁰⁾. Prothrombin time was determined using standard thromboplastin method⁽¹¹⁾.

Markers of hepatitis virus: HCV antibodies were detected using a third generation enzyme-linked immunosorbent assay (Sorin Biomedica Diagnostics, Italy),⁽¹²⁾. Serological assay for HBV markers (HbsAg and anti-HBc) were performed by a direct non-competitive sandwich assay (DiaSorin, Italy) based on ELISA technique⁽¹³⁾.

Kidney Function Tests: Colorimetric techniques were used for the determination of serum creatinine using alkaline picrate method⁽¹⁴⁾ and serum urea using Berthelot reaction⁽¹⁵⁾.

Blood picture: was done on Coulter Counter T890, (Coulter Counter, Harpenden, UK)⁽¹⁶⁾.

Cytokine measurements: An immunoenzymometric assay for quantitative measurements of human IL-6⁽¹⁷⁾ and IL-1 beta⁽¹⁸⁾ in serum were used, and the kits were supplied from BioSource International (Rue de l'Industrie 8, B-1400 Nivelles, Belgium).

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR): Qualitative estimation of HCV RNA in serum was detected by nested RT-PCR assay. Using QIA amp RNA mini kits (QIAGEN Inc.USA). Total RNA was extracted from serum samples according to the manufacturer's instructions using QIA amp. The RNA was reverse-transcribed (total volume, 40 μ l) using oligo-dNTP 100mM (Stratagene) and reverse transcriptase (Biotex). Reverse transcribed RNA(cDNA) was stored at -80 °C until used for PCR amplification. For PCR amplification, 5 μ l aliquots of 1:20 diluted reverse transcription mixture from all subjects were made up to 40 μ l in reverse transcription PCR buffer containing 10 pmol/ μ l of each primer (DiaSorin) and 5 U of Taq polymerase (Stratagene). Samples were overlaid with

mineral oil, and transferred to a Biometra thermal cycler then subjected to 34 cycles of denaturation (94°C for 1 min), annealing (50° C for 1 min), and extension (72° C for 2 min). The sequence of the primers for HCV PS000c (DiaSorin). Primers used were as follows: 5/-GGTGACGGTCTACGA GACC TC-3/, 5/ACTACTGTCTTCACGCAGA A3/- 5/GCGACCCAACACTACTGGG CT3/ 5/ATGGCGT TAGTATGAGTG3⁽¹⁹⁾. The expected product sizes were 266 bp. The PCR products (5 μ l) were loaded on a 2% agarose gel ethidium bromide and visualized by ultraviolet fluorescence.

Liver biopsy: Liver biopsy samples were obtained for diagnostic purposes percutaneously, in some cases HCC was diagnosed guided by ultra scan using a Toshiba SSA 240, an apparatus with a 3.5 MHz probe.

STATISTICAL ANALYSIS

The data were presented as mean \pm SD. Statistical analysis was performed by the statistical software SPSS 11 using independent-sample *t* test and The correlations were analyzed by the Spearman rank—order correlation coefficient. *P*<0.05 was considered statistically significant.

RESULTS

Table (1) demonstrates the results of different biochemical parameters carried out in this study including different liver function tests, kidney function tests as well as different parameters of blood picture. The results of this study showed a significant increase in ALT (34.9 \pm 12.3U/L) and AST (47.0 \pm 12.9U/L) levels in patients with HCC (Group III) than control group and HCV infected patients without liver cirrhosis (Group I). As regard serum total bilirubin level there is significant increase in serum total bilirubin in patients with HCC (4.73 \pm 1.76mg/dL) than HCV infected patients with and without liver cirrhosis (LC) (2.66 \pm 1.6, 0.85 \pm 0.260 mg/dL) as well as the control group (0.87 \pm 0.23 mg/dL).

Serum albumin level showed significant decrease in patients with HCC (1.22 \pm 0.66 g/dL) than HCV infected patients with and without

LC (2.26±0.97, 3.6±0.61g/dL) respectively but there is significant increase in PT in patients with HCC (18.62±4.35 seconds) than HCV infected patients without LC (13.5±1.29 seconds). Blood urea and serum creatinine as well as RBCs, WBCs and platelet count showed no significant changes between the different studied groups.

Serum IL-1 β level:

The serum levels of IL-1 β was significantly elevated in-group I (HCV infected patients without LC) (146.2 ± 39.9 pg/mL), group II (HCV infected patients with LC) (203.7±50.6 pg/mL), and group III (HCV infected patients with HCC) (398.4 ±50.9 pg/mL) compared with control group (71.3± 5.0 pg/mL), p= 0.001. The serum levels of IL-1 β was significantly elevated in-group III, compared with both group I and group II (t=10.81, p<0.05) (t=16.46, p<0.05) and between group II & group I (t=3.49, p <0.05) (table 2).

In both group II and group III the levels of serum IL-1 β showed a significant correlation with albumin (r = -0.348, p<0.05) (r = -0.453, p<0.05), PT (r=0.350, p<0.05) (r = 0.740, p<0.05)

respectively, and with serum bilirubin in-group III (r =0.820, p<0.05) (table 3, 4,5),

Serum IL-6 level:

The serum levels of IL-6 was significantly elevated in-group I (51.97± 17.97 pg/mL), group II (71.55±22.87pg/mL), and group III (85.43± 18.61pg/mL) compared with control group (8.87 ± 3.09pg/mL), p= 0.001. The serum levels of IL-6 was significantly elevated in-group II and group III compared with group I (t=-3.02, p<0.05) (t=5.98, p<0.05) but there was no significant difference between group II & group III (t=1.89, p >0.05)(table 2).

In both group II and group III the levels of serum IL-6 showed a significant correlation with albumin (r=0.521, p<0.05) (r=-0.762, p<0.05), PT (r=0.476, p<0.05) (r=, 0.813, p<0.05) respectively, and with serum bilirubin in group III, (r= 0.610, p<0.05) (table 3, 4,5), But there was no significant Correlation between IL- 1 β and IL-6 in the all groups (table 6).

Table (1): Comparison of mean of studied variables among studied groups (Mean±SD)

Parameters	Control	Group I	Group II	Group III
Total Bilirubin (mg/dL)	0.87±0.23	0.85±0.260	2.66±1.6*†	4.73±1.76*†‡
AST (U/L)	13.1±1.97	15.4 ±2.52	46.3±13.31*†	47.0±12.9*†
ALT (U/L)	13.3±1.94	17.0 ±2.63	31.1±6.95*†	34.9±12.3*†
Albumin (g/dL)	4.08±0.51	3.6 ±0.61	2.26±0.97*†	1.22±0.66*†‡
PT (seconds)	13.2± 1.0	13.5±1.29	16.42±2.4	18.62±4.35*†
Urea (mg/dL)	26.6±4.59	30.7±7.34	35.2±9.18	31.7±9.42
Creatinine (mg/dL)	0.7±0.33	0.79± 0.22	1.2±0.26*	1.17±0.34*
Hb (g/dL)	12.65±1.71	12±1.73	10.42±1.48	10.01±1.73
RBCs (Million/cmm)	4.64±0.43	4.46±0.46	4.53±0.52	4.24±0.46
WBCs (thousand/cmm)	6.75±1.72	6.42±1.4	7.17±2.65	8.20±3.33
Platelets (Thousand/cmm)	259.5±79.55	251.5±91.39	243.1±87.57	204.5±83.02

* p<0.05 versus control

† p<0.05 versus group I

‡ p<0.05 versus group II

Table (2): Statistical difference in serum IL-1 β and IL -6 among different groups (Mean± SD):

	Control	Group I	Group II	Group III
IL-6 (pg/mL)	8.87± 3.09	51.97± 17.97*	71.55±22.8* †	85.43 ± 18.61*†
IL-1β (pg/mL)	71.3±5 .0	146.2 ±39.9 *	203.7±50.6 *†	398.4 ±50.9*†‡

* p<0.05 versus control † p<0.05 versus group I ‡ p<0.05 versus group II

Table (3): Correlation between IL- 1 beta, IL-6 and studied variables in group I

Correlations	IL- I beta		IL -6	
	r	P	r	P
PT	0.168	0.492	0.228	0.348
AST	0.090	0.715	0.170	0.487
ALT	0.057	0.818	0.039	0.570
Total Bilirubin	0.090	0.716	0.041	0.868
Albumin	0.046	0.851	0.145	0.555

Table (4): Correlation between IL- 1 beta, IL-6 and studied variables in group II

Correlations	IL - I beta		IL -6	
	r	P	r	p
PT	0.350	<0.05 *	0.476	<0.05 *
AST	0.112	0.648	0.306	0.202
ALT	0.268	0.267	0.301	0.029
Total Bilirubin	0.244	0.314	0.351	0.141
Albumin	-0.348	<0.05 *	-0.521	<0.05 *

Table (5): Correlation between IL-1 beta, IL-6 and studied variables in group III

Correlations	IL - I beta		IL -6	
	r	P	r	P
PT	0.740	<0.05*	0.813	<0.05 *
AST	0.260	0.283	-0.085	0.730
ALT	0.070	0.776	0.321	0.018
Total Bilirubin	0.820	<0.05 *	0.610	<0.05 *
Albumin	-0.453	<0.05 *	-0.762	<0.05 *

Table (6): Correlation between IL- 1 β and Il-6 in the all groups.

IL-1 β / IL-6	r	P
Group I	0.122	0.608
Group II	0.387	0.0922
Group III	-0.0768	0.748
Control Group	0.233	0.322

DISCUSSION

Hepatitis C virus (HCV) is a common cause of hepatocellular injury that is associated with complex and vigorous immunologic mechanisms. Both humoral and cell-mediated immune responses participate in the host defence against HCV infection⁽²⁰⁾. A characteristic feature of HCV infection is a high frequency of persistence and progression to chronic liver disease (CLD). Persistent infection upsets the balance between immunostimulatory and inhibitory cytokines, which can prolong inflammation and lead to necrosis, fibrosis, and CLD⁽²¹⁾. Patients with chronic HCV infection seem destined for progression from milder forms of hepatitis to cirrhosis and, eventually, to HCC⁽²²⁾. Elevated concentrations of cytokines also represent a characteristic feature of CLD regardless of underlying aetiology, which may represent a consequence of liver dysfunction instead of inflammatory disorder⁽²³⁾.

Many works have reported high serum levels of IL-6 in various liver diseases, such as acute hepatitis⁽²⁴⁾, alcoholic cirrhosis⁽²⁵⁾, primary biliary cirrhosis (PBC)⁽²⁶⁾, chronic hepatitis and HCV-correlated liver cirrhosis⁽²⁷⁾ and in hepatocellular carcinoma (HCC)⁽²⁸⁾. On the other hand, IL-1 β gives rise to the cascade of the inflammatory response and recent reports have shown that its levels are higher in HCV-related liver diseases than in other forms of liver damage⁽²⁹⁾. Its polymorphisms behind are related to the risk of progression to HCC⁽²⁾.

In the present study, liver function tests (ALT, AST, serum total bilirubin, and PT) were significantly elevated in HCV infected patients with LC and the highest levels were reported in HCV infected patients with HCC, while there was non significant difference between HCV infected patients without LC and control group. Conversely serum albumin level significantly decreased in HCV infected patients with HCC than those with and without (LC).

These results were in agreement with the finding of Lopez et al.,⁽³⁰⁾ who founded that with the exception of bilirubin, the liver function tests were abnormal more frequently in HCC than in chronic hepatitis and cirrhosis. Also Fahim et al.,⁽³¹⁾ founded that. AST/ALT and direct/ indirect bilirubin ratios were highest in HCV infection with decompensated LC. Serum

total protein and albumin levels showed the highest reduction concomitantly with the highest increase in gamma globulin level in HCV infection with decompensated LC than those without LC. In contrast Wahib et al.,⁽³²⁾ founded that in the HCV-infected patients, the levels of ALT, AST and total bilirubin were elevated, however serum levels of albumin, and total protein were within the normal range.

In general, chronic hepatitis C patients with elevated ALT levels and high HCV RNA titres in the sera are considered to have active HCV replication in the liver and to be at risk for continued liver injury in a clinical basis. While Puoti et al.,⁽³³⁾ stated that clinical and virological features of HCV infected patients did not differ between subjects with ALT flares and those with persistently normal ALT. However, a number of recent studies showed ambivalent results in the relationships among the degree of histological damage, serum ALT level and HCV RNA titres in chronic hepatitis C⁽³⁴⁾.

Interleukins play a major role in the development of the inflammatory process, fibrosis and the regeneration of the liver. A very important role in the escalation of the inflammatory process is played by the proinflammatory cytokines IL-1, IL-4, and IL-6⁽³⁾

The result of the present study showed that serum levels of IL-1 β were significantly elevated in all patients with HCV infection compared to control group. A significant elevation in serum levels of IL-1 β were founded in HCV infected patients with LC than those without LC. However the highest levels were reported in HCV infected patients with HCC.

These results were in agreement with the finding of Lapinski⁽¹⁾ who founded that the levels of serum IL-1 β in all HCV patients were higher in comparison with healthy adults. Also, Huang et al.,⁽²⁷⁾ founded that serum IL-1 β levels were significantly elevated in all HCV infected patients compared with control group, and its levels in patients with LC or HCC were higher than that in patients without LC. Gramantieri et al.,⁽³⁵⁾ and Furusyo et al.,⁽³⁶⁾ maintain that high levels of IL-1 β result in a lengthening of the inflammatory process in the liver and high replication of the HCV. Also Farinati et al.,⁽³⁷⁾

demonstrate that as HCV-related liver damage progresses, IL-1 β levels increase, which may be relevant to liver carcinogenesis.

Interleukin-6 is multifunctional cytokine produced by a range of cells and plays a central role in host defence mechanism and modulation of immune response; it is increased in patients with different categories of liver disease⁽³⁸⁾. The results of the present study showed that serum levels of Interleukin-6 was significantly higher in all HCV infected patients compared with control group, and its levels were significantly higher in HCV infected patients with HCC and those with LC than those without LC. Interleukin-6 levels in HCV infected patients with HCC were higher than those with LC but the elevation was not statistically significant.

These findings were in agreement with the results of other studies e.g., Malaguarnera et al.,⁽³⁹⁾; Goydos et al.,⁽⁴⁰⁾; Oyanagi et al.,⁽⁴¹⁾; Huang et al.,⁽²⁷⁾; Genesca et al.,⁽⁴²⁾; Lapinski⁽¹⁾ Chau et al.,⁽⁴³⁾ and Giannitrapani et al.,⁽²⁸⁾. They showed that serum IL-6 levels are higher in patients with chronic HCV infection in comparison with healthy adults. Also Soresi et al.,⁽⁴⁴⁾ founded that IL-6 levels in patients with LC-associated HCC are higher than those in LC patients alone and control group, suggesting an increased production of this cytokine by tumour cells. Interleukin-6 production by tumour cells might also contribute to systemic complications such as induction of cachexia in the host⁽²²⁾ and local immunosuppression rather than immunopotentialiation⁽⁴⁴⁾.

In contrast, Zekri et al.,⁽²¹⁾ and Tovey et al.,⁽⁴⁵⁾ showed that IL-6 was slightly higher only in asymptomatic HCV carriers than controls, but apparently normal in both HCC and CLD patients,

In the present study, both IL-1 β , IL-6 showed a strong correlation with serum albumin, PT in patients with LC and patients with HCC. Also their levels significantly correlate with serum total bilirubin in patients with HCC. But there was no significant correlation with ALT, AST in all groups. These results were in agreement with the finding of Huang et al.,⁽²⁷⁾; who founded that the serum concentrations of IL-1 β and IL-6 correlated better with indices of hepatic

dysfunction (PT and albumin) than with parameters of hepatic inflammation (ALT, AST). Also Genesca et al.,⁽⁴²⁾ reported that increased level of IL-6 is correlated with the stage of disease in liver cirrhosis. Malaguarnera et al.,⁽³⁹⁾ demonstrate that higher levels of IL-6 correlated with tumour size and cancer aggressiveness in patients with HCC. These results further support the role of IL-1 β in activation of the sinus cells of the endothelium and production of acute phase proteins such as ceruloplasmin and C3 component complement in the liver⁽⁴⁶⁾.

CONCLUSION

Serum IL-1 beta, and IL-6 levels are elevated in patients with hepatitis C-related liver diseases, especially in LC and HCC patients. Their levels reflect hepatic dysfunction better than liver inflammation parameters, accordingly; we may use serum IL-1 beta and IL-6 as markers for Liver disease progression in HCV-infected cases instead of invasive techniques. Further studies on the role of IL-1 beta, and IL-6 gene polymorphism in HCV patients are recommended.

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