Research Article

Serum Interleukin-١٨ in Hepatitis C Virus Infection

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Abstract
Background: Proinflammatory cytokines, including interleukin-١٨ (IL-١٨) play a critical role in antiviral immune responses and in liver injury induced by viral infection including hepatitis C viral infection. The aim of this study was to clarify the significance of IL-١٨ in regard to the degree of liver inflammation in hepatitis C viral infection and HCV- associated chronic hepatitis by comparing serum IL-١٨ levels with liver enzymes and liver biopsy. **Methods:** This prospective study included eighty eight (٨٨) patients; sixty eight (٦٨) patients were positive for HCV and twenty (٢٠) healthy control subjects. They were divided into three main groups; group I (composed of ٣٤ patients with HCV infection with persistently normal liver enzymes (ALT, AST) for ٦ months, and group II (Composed of ٣٤ patients with HCV infection with elevated liver enzymes (ALT, AST) (chronic hepatitis C), and group III (Composed of ٢٠ apparently healthy subjects). Ten ml of venous blood was withdrawn by sterile venipuncture and used for routine investigations including, complete liver function tests, HCV antibodies by ELISA and HCV-RNA by RT-PCR and IL-١٨ serum level by ELISA technique. **Results:** There was highly significant increased level of IL-١٨ in group II (chronic hepatitis c) when compared with both group I and III (P-value < ٠.٠٠٣ & < ٠.٠٠١ respectively). Moreover, group II showed highly statistically significant increased ALT and AST when compared to both group I and group III (P-value = ٠.٠٢ & < ٠.٠٠١ respectively). HCV RNA viral load in group II was statistically increased when compared to group I (p-value = ٠.٠٤). Strong significantly positive correlation between IL-١٨ in group III and AST level (r = ٠.٣٥, p- value = ٠.٠٤), and a non-significant positive correlation between ALT and HCV-RNA levels (r = ٠.٤, p-value = ٠.٠١). While group I showed highly significant positive correlation between levels of serum IL-١٨ and HCV-RNA (r = ٠.٦, p-value < ٠.٠٠١) but significantly negative correlation between serum IL-١٨ and AST (r = - ٠.٣٩, p-value = ٠.٠٢). Moreover, there was a positive relation between degree of fibrosis and serum level of IL-١٨ in HCV patients. **Conclusion:** These data confirm previous studies of enhanced serum IL-١٨ in HCV patients and suggest its marked increase in chronic hepatitis c patients compared to HCV infected patients and controls. So our data suggest that IL-١٨ can be used as a non-invasive marker for detection of the chronicity and severity of liver inflammation in chronic hepatitis C.

Keywords: HCV infection, viral hepatitis, IL- ١٨, cytokines

Introduction
Hepatitis C is an infectious disease affecting primarily the liver, caused by the hepatitis C virus (HCV). The infection is often asymptomatic, but chronic infection can lead to scarring of the liver and ultimately to cirrhosis[١٢]. Viral infections are known to suppress the immune system; induction of IL-١٨ binding protein and inhibition of IL-١٨[٨]. About ٢٠٪ of chronically infected patients develop cirrhosis with enhanced risk of hepatocellular carcinoma, because of the difficulty in eradicating HCV[١٣]. Most of the available studies revolve around estimation of serum ALT levels and liver biopsy to establish relationship between the two most commonly used parameters to assess progression of underlying liver disease in chronic hepatitis C. These studies showed a strong correlation between persistently elevated serum ALT levels and severity of liver disease proved by liver biopsy. These studies further provided evidences that individuals with normal alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels may have significant liver disease[٤]. IL-١٨ generates cell-mediated immune responses via activation of

Th١ T-helper cell sub-populations primarily through the induction of interferon-gamma. There are few data on IL-١٨ in relation to infectious diseases, but it plays a prominent role in chronic HCV infection[(١١)].

In chronic hepatitis C virus infection, a significant up regulation of IL-١٨ occurs in the inflammatory infiltrate, suggesting a role of this cytokine in the chronic cellular immune response to hepatocytes in the course of the disease. The aim of this study was to clarify the significance of IL-١٨ in regard to the degree of inflammation in HCV infection and HCV-associated chronic hepatitis by comparing serum IL-١٨ levels with liver enzymes and liver biopsy.

Subjects and Methods

This prospective study was conducted at department of Clinical Pathology, El-Minia University Hospital in the period between April, ٢٠١٠ and September, ٢٠١٠. Eighty eight (٨٨) subjects were included in the study sixty eight (٦٨) patients were positive for HCV antibody by third generation enzyme-linked immunosorbent assay (ELISA) and confirmed by HCV-RNA detection by real time PCR and twenty (٢٠) healthy control subjects.

They were classified into three main groups; group I (composed of ٣٤ patients with HCV infection with persistently normal liver enzymes (ALT, AST) for ٦ months their ages ranged from ٢٠ years to ٥٧ years (١٩ males, ١٥ females) and group II (Composed of ٣٤ patients with HCV infection with elevated liver enzymes (ALT, AST) (chronic hepatitis C), their ages ranged from ٢١ years to ٥٦ years (٢٤ males, ١٠ females), and group III (Composed of ٢٠ apparently healthy subjects their ages ranged from ٢٢ years to ٥٦ years (١٥ males, ٥ females). The study was conducted under the ethical aspect, according to ethics committee of faculty of medicine, El-Minia University; all patients have given a written consent as regard the participation in the study and having the right to withdraw from the study. Patients with liver diseases other than HCV, or other medical diseases e.g. hypertension, diabetes mellitus, endocrinal disorders or neurological diseases or receiving antiviral drugs were excluded from this study.

All three groups were subjected to careful history taking, Physical examination, abdominal ultrasound, liver biopsy. Ten ml of venous blood was withdrawn by sterile venipuncture and used for routine investigations (complete liver function tests, HCV antibodies by ELISA and HCV-RNA by real time PCR) and IL-١٨ serum level by ELISA using kits supplied by Bender Med Systems GmbH (Vienna, Austria) and reagents for human IL-١٨ ELISA BMS٢٦٧/٢ (٩٦ tests).

Statistical analysis

All data were analyzed using the statistical package SPSS (version ١٢.٠, SPSS Inc., Chicago, IL). Statistical analysis was performed using t-test, analysis of variance (ANOVA), ٢-tailed Fisher's exact test for comparisons of qualitative data, and Spearman's coefficient for correlations (r) of quantitative data, when appropriate. Results are expressed as means ± SD or frequency. A p-value less than ٠.٠٥ was considered to be significant.

Results

All obtained results of different groups were summarized in tables from ١ to ٥ and figures from ١ to ٤. There was statistically significant difference when comparing group II to both group I and group III regarding their age (P-value = ٠.٠٢ & = ٠.٠١ respectively), while there was no statistically significant difference among three groups when compared with each other (P-value = ٠.٥, = ٠.٥ & = ٠.١ respectively) as shown in table ١. When comparing group I with group III there was no statistically significant difference between both groups regarding AST level (P-value = ٠.١٧٨) but there was highly statistically significant increase when comparing group II to both group I and group III (P-value < ٠.٠٠١).

In addition, when comparing group I with group III there was no statistically significant difference between both groups regarding ALT level (P-value = ٠.٤) but there was highly statistically significant increase when comparing group II to both group I and group III (P-value < ٠.٠٠١). Also there was highly statistically significant increased ALP level in group I and II when compared to group III (P-value < ٠.٠٠١) and also when comparing both groups with each other (P-value = ٠.٠٢) as shown in table ٢. when comparing group I and

group II with group III there was statistically significant decreased albumin level in both groups I and II (P-value = ٠.٠٠٤ & = ٠.٠٠١ respectively). While there was highly statistically significant difference in albumin level when compared group I with group II (P-value < ٠.٠٠٠١), there was no statistically significant difference between three groups when compared with each other (P value = ٠.٢, = ٠.٢, = ٠.٧ respectively). Also there was no statistically significant difference between three groups when compared with each other (P-value = ٠.٧, = ٠.٤ & = ٠.٧ respectively). When comparing both group I and II with group III there was statistically significant increase of PT (P-value = ٠.٠٢, & = ٠.٠٠٢ respectively).

While there was highly statistically significant increase of PT when compared group II with group III (P-value = ٠.٠٠٢). Meanwhile, there was highly statistically significant decrease of PC in both groups I and II when compared with group III (P value < ٠.٠٠٢, & < ٠.٠٠١ respectively) and also there was significant statistical difference between group I and group II when compared with each other (P value = ٠.٠٥). There was highly statistically significant increased INR in both groups I and II when compared with group III (P-value < ٠.٠٠٢ & < ٠.٠٠١ respectively). Also there was statistically significant difference in INR when comparing both groups I and II each other (P-value = ٠.٠٣٧) as shown in table ٣. When comparing group I with group III there was no statistically significant difference between both groups as regarding IL-١٨ level (P value = ٠.١٥), while there was highly statistically significant increased level of IL-١٨ when comparing group II with both group I and III (P-value < ٠.٠٠٣ & < ٠.٠٠١ respectively).

Moreover, there was statistically significant increased level of HCV- RNA viral load in group II when compared to group I (P-value = ٠.٠٤) as shown in table ٤. Moreover, there was a positive relation between degree of fibrosis and serum level of IL-١٨ in HCV patients as shown in table ٥. There was strong significant positive correlation between IL-١٨ and PCR in group I (r = ٠.٦٠, p-value < ٠.٠٠١) as shown in fig.١. However, non-significant positive correlation was found between serum IL-١٨ and PCR in group II (r = ٠.٣, p-value = ٠.١٣) as shown in fig.٢.

Table ١: Demographic data of different studied groups.

Variables		Group I n=٣٤	Group II n=٣٤	Group III n= ٢٠	P-value Group I Vs III	P-value Group II Vs III	P-value Group I Vs II
Age (years)	Range	٢٠-٥٧	٢١-٥٦	٢٢-٥٦	٠.٦	٠.٠١	٠.٠٢
	Mean ± SD	٣٥.٥ ± ٨.٨	٤٠.٨ ± ٨.٧	٣٤.٤ ± ٨.٥			
Sex	*Male No (%)*	١٩ (٥٥.٩)%	٢٤ (٧٠.٥)%	١٥ (٧٥)%	٠.٤	٠.١	٠.١
	Female NO (%)	١٥(٤٤.١)%	١٠(٢٩.٥)%	٥(٢٥)%	٠.٥	٠.٥	٠.١

Table ٢: Comparison between different studied groups regarding AST, ALT and ALP.

Variables		Group I n=٣٤	Group II n=٣٤	Group III n= ٢٠	P-value Group I Vs III	P-value Group II Vs III	P-value Group I Vs II
AST (U/L)	Range	١٥ - ٤٣	٤٠ -١٧٦	١٥ - ٣٩	٠.١٧٨	٠.٠٠١	٠.٠٠١
	Mean ± SD	٢٧.٢ ± ٦.٧	٧٢.٨ ± ٣٠	٢٤.٨± ٥.٨			
ALT (U/L)	Range	١٨ - ٤٢	٤٠ -١٩٢	١٨ -٣٩	٠.٤٥٣	٠.٠٠١	٠.٠٠١
	Mean ± SD	٢٧.٥ ± ٦.٤	٧٩.١± ٣٧.٨	٢٨.٨ ± ٥.٤			
ALP (U/L)	Range	١٢٣ - ٢١٢	١٢٥ - ٢٨٠	١١٠ -١٣٥	٠.٠٠١	٠.٠٠١	٠.٠٢١
	Mean ± SD	١٦٧ ± ٢٢.٧	١٨١ ± ٢٧.٠	١٢٧ ± ٧.٩			

Table ٣: Comparison between different studied groups regarding mean values of albumin level, total and direct bilirubin, prothrombin time concentration (%) and INR.

Variables		Group I n=٣٤	Group II n=٣٤	Group III n= ٢٠	P-value Group I Vs III	P-value Group II Vs III	P-value Group I Vs II
Albumin (gm/dl)	Range	٣ - ٤.٨	٢.٦ - ٤.٤	٤.٠ - ٥.٢	٠.٠٠٤	٠.٠٠١	٠.٠٠٠١
	Mean±SD	٤.٢ ± ٠.٤	٣.٧ ± ٠.٤	٤.٦ ± ٠.٤			
Total Bil (mg/dl)	Range	٠.٦ - ١.٣	٠.٦ - ٠.٩	٠.٦ - ١	٠.٢	٠.٢	٠.٧
	Mean±SD	٠.٧٤± ٠.١٢	٠.٧٥±٠.٠٦	٠.٧٨ ± ٠.١			
D Bil (mg/dl)	Range	٠.١- ٠.٢٥	٠.١- ٠.٢	٠.١ - ٠.٢	٠.٧	٠.٤	٠.٧
	Mean±SD	٠.١٧ ±.٠٤	٠.١٨ ± .٠٣	٠.١٧ ±.٠٤			
Prothrombin time (sec)	Range	١٢.٧ - ١٧.٩	١٣.٣–٢٢.٨	١٢.٢ - ١٤.٢	٠.٠٢	٠.٠٠٢	٠.٠٣*
	Mean±SD	١٤.٧ ± ١.٢	١٥.٦ ± ٢.٣	١٢.٩ ± ٠.٧			
Prothrombin conc (%)	Range	٥٦٪–٩٦٪	٣٦٪–١٠٠٪	٦٨٪– ٩٨٪	٠.٠٠٢	٠.٠٠١	٠.٠٥
	Mean±SD	٨٠.٤±١١.٢	٧٣.٤±١٦.٥	٨٩.٩ ± ٩.١			
INR	Range	١.٠ – ١.٦	١.٠ – ٢.٦	١.٠-١.٢	٠.٠٠٢	٠.٠٠١	٠.٠٣٧
	Mean±SD	١.٢± .١٦٩	١.٣٧±٠.٣٥	١.٠٩±٠.١٢٥			

Table ٤: Comparison between different studied groups regarding, mean values serum IL-١٨ and the viral load.

Variables		Group I N = ٣٤	Group II N = ٣٤	Group III N = ٢٠	P-value group I Vs III	P-value group II Vs III	P-value group I Vs II
IL-١٨ (pg/ml)	Range	٠ - ١٠٣٧	١٠.٤ - ١٧٧٨	٠ - ١٥١	٠.١٥٦	٠.٠٠١**	٠.٠٠٣**
	Mean± SD	١٢٦.٩ ± ٢٣٧.٧٨	٣٧١.٧٣±٤٣٩.٤٥	٢٦.٧±٥٥.٢			
Viral load (IU/ml)	Range	٤٢١ - ٩٠٠٠٠٠	٣٥٩- ١٨٠٠٠٠٠	---	….		
	Mean±SD	٢٧٠٠٠٠±٣٧٠٠٠٠	٤٨٠٠٠٠±٥٤٠٠٠٠			….	٠.٠٤*

*** Significant** **** Highly significant**

Table (٥): The relation between IL-١٨ and degree of fibrosis in patients groups

Stage of Fibrosis (F)	No. of Patients	Level of IL -١٨(pg/ml)	
		Range	Mean ± SD
F٠	٧	٣٨-٣٩٤	١٦٣.٥ ±١٤٦.٢
F١	٢٠	٠-١٠٣٧	٢٩٢.٧± ٢٠٠.٦
F٢	٢١	٠-١٧٧٨	٤١٠.٣ ± ٢٢٢.٥
F٣	١٨	٠-١٢٧٥	٣٢٤± ١٩٠.١
F٤	٢	١٣١-٨١٥	٤٨٣ ± ٤٧٣.٢

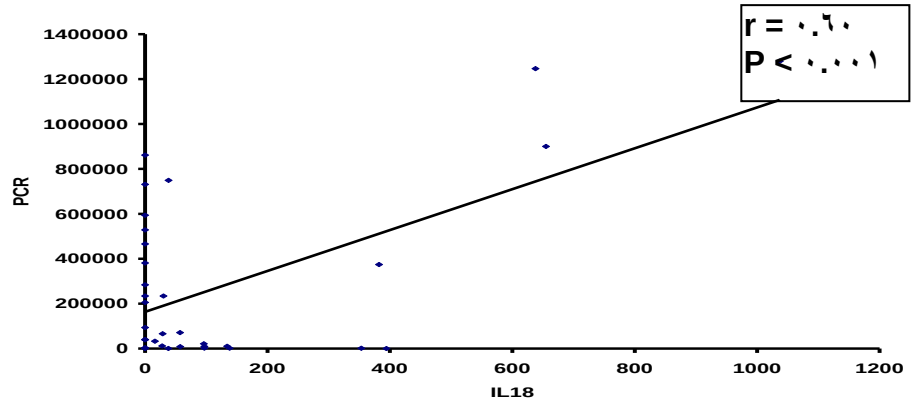

Fig. ١: Correlation between serum IL-١٨ and HCV-RNA viral load in group I.

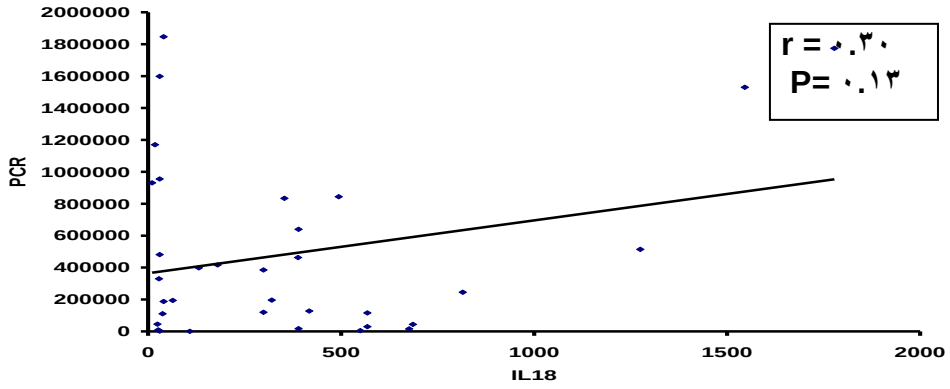

Fig. ٢: Correlation between serum IL-١٨ and HCV-RNA viral load in group II.

There was strong significant positive correlation between serum IL-١٨ and HCV-RNA viral load in group I (r =٠.٦٠, P-value < ٠.٠٠١). However, non significant positive correlation was found between serum IL-١٨ and HCV-RNA viral load in group II (r = ٠.٣, P-value = ٠.١٣).

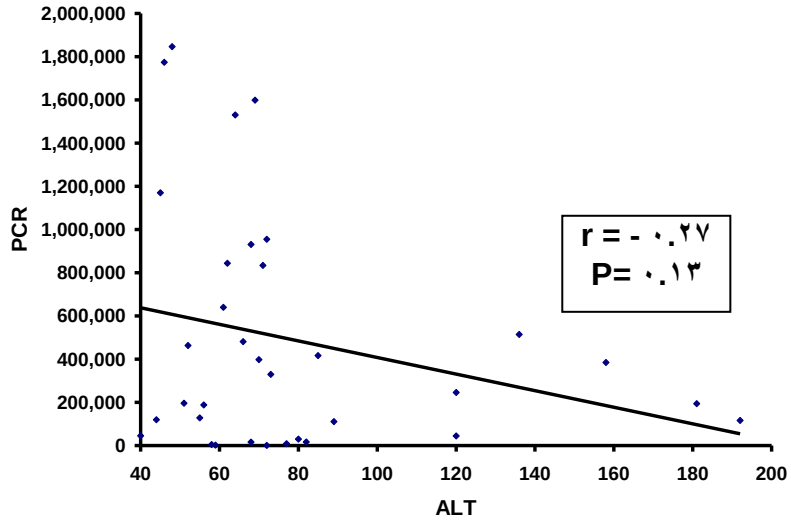

Fig. ٣: Correlation between serum ALT and HCV-RNA viral load in group I.

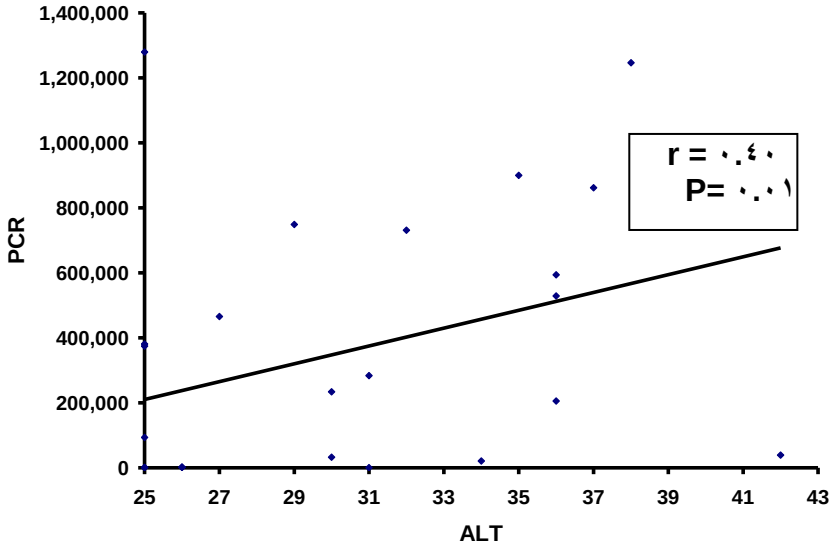

Fig. ٤: Correlation between serum ALT and HCV-RNA viral load in group II.

There was non significant negative correlation between ALT and results of HCV-RNA viral load in group I (r = -٠.٢٧, P-value =٠.١٣) but, significant positive correlation was found between ALT and results of HCV-RNA viral load in group II (r = ٠.٤٠, P-value = ٠.٠١).

Discussion

Hepatitis C virus infection is a disease with a significant global impact; it is the most common liver disease worldwide[١٢]. In patients with chronic viral hepatitis, liver biopsy is the traditional gold standard method to establish the diagnosis. The biopsy stage (degree of fibrosis) and grade (inflammatory activity) predict the course of the disease (progression to cirrhosis) and response to interferon (IFN) therapy. However this procedure has many disadvantages, it is invasive, costly and difficult to standardize[١]. Cytokines are recognized as an important factor in the pathophysiology of chronic hepatitis C. IL-١٨, previously known as interferon-gamma-inducing factor, is a pleiotropic pro-inflammatory cytokine that is expressed mainly by peripheral blood mononuclear cells and macrophages.

In chronic hepatitis C virus infection, a significant up-regulation of IL-١٨ occurs in the inflammatory infiltrate, suggesting a role of this cytokine in the chronic cellular immune response to hepatocytes in the course of the disease. The present study showed that, serum levels of IL-١٨ were significantly increased in chronic HCV patients than in healthy controls. Also the present study showed no statistically significant difference between group I when compared with group III as regarding IL-١٨ level (p value = ٠.١٥*)*.

While highly statistically significant level of IL-١٨ in group II when compared with both groups I and III (p value ≤٠.٠٠٣ & ≤٠.٠٠١ respectively*)*, and this explained that group I with normal enzymes not associated with viral activity that accompanied with proinflammatory expression of IL-١٨. These findings were in agreement with that reported by El-Kady et al.,[٥] who found a significantly higher serum levels of IFN-γ and IL-١٨ in patients infected with HCV compared to controls. The present study showed significant negative correlation between serum IL-١٨ and AST in group I (r = -٠.٣٩, P-value = ٠.٠٢).

However there was significant positive correlation between serum IL-١٨ and AST in group II (r = ٠.٣٥, P-value = ٠.٠٤), and this is in agreement with some authors who suggested that the serum aminotransferases, especially the AST level, was associated with liver damage[١٥], explaining more release of AST from liver cell injury in group II. In agreement with the present study results Hongyu et al.,[٧] showed a positive correlation between serum levels of IL-١٨ and ALT in patients of hepatitis C virus infection. While Butt et al.,[٣] found a positive association between ALT activity in the serum and duration of HCV infection. As regard to albumin the present study showed no correlation between serum IL-١٨ and albumin in group I (r = - ٠.٠٣, P-value = ٠.٩).

However, non significant negative correlation was found between serum IL-١٨ and albumin in group II (r = - ٠.٢٩, P-value = ٠.٠٩) and this may suggest that IL-١٨ may be a sensitive marker for early liver affection, however albumin is more related to chronicity and cirrhosis. Regarding to PCR the present study showed strong significant positive correlation between serum IL-١٨ and results of PCR in group I (r = ٠.٦, P-value < ٠.٠٠١) and this may suggest that IL-١٨ is an early marker for HCV even with normal enzymes. But, non significant positive correlation was found between serum IL-١٨ and results of PCR in group II (r = ٠.٣, P-value = ٠.١٣). The present study showed non significant negative correlation between ALT and results of PCR in group I (r = -٠.٢٧, P-value = ٠.١٣) But, significant positive correlation was found between ALT and results of PCR in group II (r = ٠.٤, P-value = ٠.٠١). These were in agreement with that reported by Ghany et al.,[٦] who suggest that immunosuppression results in higher HCV RNA but lower ALT levels in chronically HCV infected patients. Bozdayia et al.,[٢] have reported higher ALT levels in patients with high viral load and this explained by viral cytopathic activity and or immune mediated process. But Butt et al.,[٣] has suggested that no significant difference in viral load exists between patients with abnormal ALT levels and those with normal ALT levels. However Zechini et al.,[١٥] had shown that virologic characteristics do not differ from patients with normal or increased liver enzymes together with no difference in distribution of genotypes among affected patients.

These differences explained by Wedemeyer et al.,[١٤] who observed that the dramatic fluctuation of ribonucleic acid of HCV (HCV-RNA) levels in patients with HCV may have a temporal relation with the ALT fluctuation, which in turn may be correlated with the hepatocyte injury in infected patients with increased liver enzymes in contrast to patients with normal liver enzymes who may have a persistently stable HCV-RNA levels. In addition, Kurasaki et al.,[٩] supported the present study by showing that the ALT in the HCV RNA high level group was much higher than that in the HCV RNA low level group and indicated that HCV replication is related to the progress of chronic liver disease, and supported the theory that HCV may have cytopathogenic effect.

The present study that showed a strong correlation between serum IL-١٨ and HCV-RNA viral load and the positive relation between IL-١٨ and the degree of fibrosis detected in liver biopsy indicating that serum

IL-١٨ level can be used with the results of PCR to get an early and sensitive information about liver inflammation in chronic HCV infection which can be confirmed by liver biopsy. Cross-sectional studies on the correlation between serum HCV-RNA viral load and serum aminotransferases (ALT and AST) levels in patients with chronic hepatitis C have yielded conflicting results. Lau et al.[١٠] found no correlation between HCV viral load and serum ALT levels and this disagree with the results of the present study. These data confirm previous studies of enhanced serum IL-١٨ in HCV patients and suggest its marked increase in chronic hepatitis C patients compared to HCV infected patients and controls.

Conclusion

In conclusion we suggest that IL-١٨ can be used as a non-invasive marker for detection of the chronicity and severity of liver inflammation in chronic hepatitis C.

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