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Evaluation of Serum Level of IL-6 in Chronic Hepatitis C Virus Infected Egyptian Patients

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Abstract Background: Hepatitis C virus (HCV) infection is a worldwide public health problem. The highest prevalence is in Egypt and constitute about 10-20% of the general population. IL-6 is a plieotropic cytokine that plays a role in the acute phase response; it is released from various cells, such as, Leukocytes, fibroblasts, macrophages and endothelia cells. IL-6 is produced by kupffer cells in the liver and leads to production of the acute phase proteins. Aims: This study was conducted to evaluate serum level of IL-6 as a marker of liver fibrosis in chronic hepatitis C virus infected Egyptian patients and to assess its correlation with the stages of liver fibrosis. Methods: A case control study was conducted on 40 Egyptian patients with chronic HCV infection (group1). Studied patients were divided according to the stage of fibrosis assessed by fibroscan into two sub-groups: Group Ia: 22 Patients with early fibrosis (F0, F1, F2) and Group Ib: 18 Patients with late fibrosis (F3, F4) compared to age matched 40 healthy controls (group 11). Routine laboratory investigations, Hepatitis markers including (HBsAg), HCV antibody and HCV RNA by real time polymerase chain reaction (PCR) and, serum Interleukin-6 and Abdominal ultrasonography were done for all studied groups and assessment of liver fibrosis by APRI score & FIB-4for patients and control but Fibro scan for patients only. Results: There were highly significant Elevation of serum level of IL-6 in group1 when compared to the control and This elevation was more significant in (group Ib) when compared to (group Ia) and There was highly significant positive correlation between serum IL-6 and each of serum ALT, AST and Fibro scan in group 1. Conclusion: The study concluded that a highly significant elevation of serum level of IL-6 in chronic HCV Egyptian patients and there was a highly significant positive correlation between IL-6 and stages of liver fibrosis assessed by fibroscan.

Keywords: *HCV, IL-6, APRI score, FIB-4*

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1. Introduction

Hepatitis C virus (HCV) infection is a public health problem worldwide. It is estimated more than 185 million people around the world have been infected with it according to the world health organization (WHO) [1]. The highest prevalence is in Egypt and constitute about 10-20% of the general population [2].

Chronic hepatitis, liver cirrhosis and hepatocellula carcinoma remain as the major complications of chronic HCV infection worldwide [3].

Hepatic fibrosis is one of the chronic inflammatory liver diseases. It is a complex process that involves activation of cells which produce inflammatory mediators, cytokines, and cause tissue remodeling and deposition of extracellular matrix [4].

In chronic liver disease as chronic hepatitis C an increase in the level of pro-inflammatory cytokines has been noted and the most important of them are IL-1 (alpha and beta) and IL-6 [5].

IL-6 is a plieotropic cytokine that plays a role in the acute phase response; it is released from various cells, such as, Leukocytes, fibroblasts, endothelial cells and macrophages. IL-6 is produced by kupffer cells in the liver and leads to production of the acute phase proteins [6].

Aim of the study: was to evaluate serum level of IL-6 as a marker of liver fibrosis in chronic hepatitis C virus infected Egyptian patients and to assess its correlation with the stages liver fibrosis.

2. Patients and Methods

This study includes 40 Egyptian patients with chronic HCV infection, they were 15 males (37.5%) and 25 females (62.5%) and their ages ranged between (33-60) years with mean (46.1 ± 7.91 years) (group I), and 40 apparently healthy volunteers were included in the study as a control group (group II). They were 20 males (50%) and 20 females (50%) and their ages ranged from (23 - 64) years with mean (45.73 ± 9.61) years.

All patients were selected from the virus C treatment unit at El-Mansoura International Hospital during the period from March 2015 to December 2015after obtaining oral consent to be participated in this study. Also approval of ethical committee of faculty of medicine Al-Azhar University was obtained:

2.1. Exclusion Criteria

Co infection with HIV or hepatitis B virus (HBV), Patients with history or evidence of any malignancies, and autoimmune diseases Patients suffering from any organ failure and other causes of cirrhosis e.g. alcohol, obese, diabetic patients, and Patients on antiviral treatment or received antiviral treatment before were excluded from the study.

Methods:

All subjected in this study were subjected to the following:

Full medical history and complete physical examination.

Measurement of body mass index (BMI): weight divided by the square of the height (Kg/m2)

Laboratory investigations:

Sample collection and storage:

Five ml of venous blood were withdrawn under complete aseptic technique from the fasting patients and control in a sterile vacutainer tube 1ml for complete blood picture were taken on EDTA solution,3ml of the samples were taken in plain tube, left to clot, centrifuged at 2000×g for 10 minutes and sera were separated without delay. The routine investigations (liver function, kidney function and fasting blood sugar) were done on the same day. The rest of the serum sample were stored frozen at -20 C after careful labeling till the time of the assay

A- Complete blood picture (CBC) were done on sysmex KN21, Fasting and post prandial blood sugar, Liver function tests (Serum albumin, bilirubin, alanine transaminases (ALT) and aspartate transaminase (AST), Kidney function tests including serum urea and creatinine All of them were done on cobas 311 clinical chemistry auto-analyzer from ROCH diagnostic company and Hepatitis markers including (HBsAg), HCV antibody and HCV RNA by real time polymerase chain reaction (PCR).were done.

B-Specific investigations: Serum Interleukin-6was measured by quantitative sandwich enzyme immuonoassay technique. ELISA kit was supplied from ASSAYPRO Company with catalog Number E11006-1 [7].

3-Radiologic investigations:

A-Abdominal ultrasonography with special emphasis on:

Liver size, echo pattern and presence of focal lesion, splenic size and echo pattern, portal vein diameters, and presence or absence of ascites.

B- Non invasive assessment of liver fibrosis:

1-Fibro scan for patients only: Fibro Scan is a non-invasive device that assesses the 'hardness' (or stiffness) of the liver via the technique of transient elastography. Liver hardness is evaluated by measuring the velocity of a vibration wave (also called a 'shear wave') generated on the skin. Shear wave velocity is determined by measuring the time the vibration wave takes to travel to a particular depth inside the liver [8]. In Hepatitis C:

2 to 7 kpa means f0-f1 stage.

8 to 9 kpa means f2 stage.

9 to 14 kpa means f3 stage.

14 or higher kpa means f4 stage

The stage of fibrosis varied from 0 to 4 (F0 = no fibrosis; F1 = portal fibrosis without septa; F2 = portal fibrosis with few septa; F3 = septal fibrosis, without cirrhosis; F4 = cirrhosis (Figure14) [8].

2- APRI score:

It is an alternative method to liver biopsy with satisfactory sensitivity and specificity. it is calculated using the patient's aspartate aminotransferase (AST) level and platelet count, and the upper limit of normal of aspartate aminotransferase (AST) level.

$$APRI = \frac{\frac{AST\ Level}{AST\ (Upper\ Limit\ of\ Normal)}}{Platelet\ Count\ (10^9 / L)} X 100$$ [9].

A meta-analysis of 40 studies found that an APRI cutoff of greater than or equal to 0.7 had an estimated sensitivity of 77% and specificity of 72% for detection of significant hepatic fibrosis (greater than or equal to F2 by METAVIR). A cutoff score of at least 1.0 has an estimated sensitivity of 61% to 76% and specificity of 64% to 72% for detection of severe fibrosis/cirrhosis (F3 to F4 by METAVIR). For detection of cirrhosis, a cutoff score of at least 2.0 was more specific (91%) but less sensitive (46%).

3- Fibrosis Index-4: The FIB-4 is an easy-to-use, quick, and inexpensive test that provides results immediately. Results are generated utilizing age, AST, ALT, and platelet count [10].

$$FIB-4 = \frac{Age(years)\ x\ AST(U/L)}{Platelet\ Count\left(10^9 / L\right) x \sqrt{ALT(U/L)}}.$$

The FIB-4 index enabled the correct identification of patients with severe fibrosis (F3-F4) and cirrhosis. An FIB-4 index <1.45 had a negative predictive value of 94.7% to exclude severe fibrosis with a sensitivity of 74.3%. A FIB-4 index higher than 3.25, had a positive predictive value to confirm the existence of a significant fibrosis (F3-F4) of 82.1% with a specificity of 98.2%. Using these ranges, 72.8% of the 847 liver biopsies were correctly classified [11].

2.2. Statistical Analysis

Data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 20. Qualitative data were presented as number and percentages while quantitative data were presented as mean, standard deviations and ranges when parametric. The comparison between two groups with qualitative data were done by using Chi-square test and/or Fisher exact test was used instead of Chi-square test when the expected count in any cell was found less than 5.The comparison between two groups regarding quantitative data with parametric distribution was done by using Independent t-test..Spearman correlation coefficients were used to assess the correlation between two quantitative parameters in the same group. The confidence interval was set to 95% and

the margin of error accepted was set to 5%. So, the p-value was considered significant as the following: P > 0.05: Non significant. P < 0.05: Significant .P < 0.01: Highly significant.

ROC-curve: Receiver Operating Characteristic curve analysis

Sensitivity: Probability that the test results will be positive when the disease is present (true positive rate, expressed as a percentage).

Specificity: Probability that the test results will be negative when the disease is present (true negative rate, expressed as a percentage).

PPV: Positive Predictive value (probability that the disease is present when the test is positive).

NPV: Negative Predictive value (probability that the disease is present when the test is negative).

Accuracy: the ratio of the true positive and true negative on all patients.

3. Results

3.1. Demographic Data of Studied Groups Showed

Age of group I (40 patients with chronic HCV) ranged from (33-60) year, with mean ± SD 0f (46.1±7.91) year, BMI ranged from (19-25),with mean ± SD of (22.89±1.6), and age of group II(40 healthy volunteers as a control) ranged from (23-64) year, with mean ± SD of (45.73±9.61) year, BMI ranged from (18-25), with mean ± SD of (23.28±1.55).

According to stages of fibrosis assessed by fibroscan our Patients (group I) were classified into two sub-groups: Group Ia: Patients with early fibrosis included 22 patients They were 11 males (50%) and 11 females (50%) and their ages ranged between (35 - 57) years old, with mean (44.73± 7.3 years). 19 patient (86%) had F0-1,and 3(14%) had F2.

Group Ib: Patients with advanced fibrosis Include 18 patients. They were 4 males (22.2%) and 14 females (77.8%) and their ages ranged between (33 - 60) years old, with mean (49.3±7.7 years). 5 patients (28%) had F3 and 13(72%) had F4

There was a significant increase in the mean ± SD of serum total serum bilirubin in (Group I) (.92 ±.49) when compared to (Group II) (.75 ±.15) (P value < 0.05)). While there was a significant decrease in the mean ± SD of serum albumin and platelet count in (Group I) (3.8 ±.44) (185.03 ± 77.05) when compared to (Group II) (4.05 ±.4) (247.15± 52.65) (P value <.05),), (P < 0.001) respectively (Table 1).

Also There was a highly significant increase in the mean ± SD of serum ALT, AST, serum IL-6, APRI score and FIB-4 in (Group I) (50.65 ± 30.18), (52.28 ± 34.51), (0.91 ± 0.57) ,(1.78 ± 0.24), (3.79 ± 0.31) when compared to (Group II) (24.83 ± 8.35), (26.28 ± 9.18) (0.04 ± 0.02), (0.3 ± 0.12), (0.89± 0.33) (P < 0.001) respectively (P < 0.001), (Table 1, Figure 1) while, there was no significant differences in the mean ± SD of WBCs, RBCs, HB, in (Group I) (6.70 ± 2.04), (3.92 ±.69), (12.70 ±1.41), (103.25 ± 10.48) when compared to (Group II), (7.28 ±1.63), (4.16 ±.73), (13.21±1.46), (P >0.05) respectively.

Table 1. Comparison between (Group I) and (Group II).

	Group I (n=40)	Group II (n=40)	P-value	Sig.
WBC	6.70±2.04	7.28±1.63	0.182	NS
HB	12.70±1.41	13.21±1.46	0.116	NS
RBC	3.92±0.69	4.16±0.73	0.135	NS
PLT	185.03±77.05	247.15±52.65	<0.001	HS
ALT	50.65±30.18	24.83±8.35	<0.001	HS
AST	52.28±34.51	26.28±9.18	<0.001	HS
T Bilirubin	0.92±0.49	0.75±0.15	0.046	S
S alb	3.8±0.44	4.05±0.4	0.009	S
IL6	0.91±0.57	0.04±0.02	<0.001	HS
APRI	1.78±0.24	0.3±0.12	<0.001	HS
FIB4	3.79±0.31	0.89±0.33	<0.001	HS

Table 2. Comparison between (Group Ia) and (Group Ib)

	Group Ia (n=22)	Group Ib (n=18)	P-value	Sig.
WBC	7.40±1.67	5.86±2.41	0.022	S
HB	13.66±1.34	13.08±1.47	0.199	NS
RBC	4.89±0.46	4.64±0.50	0.115	NS
PLT	233.14±54.92	126.22±56.90	<0.001	HS
ALT	42.82±18.52	60.22±38.59	0.069	NS
AST	46.00±21.73	59.89±25.83	0.072	NS
T Bilirubin	0.73±0.33	1.15±0.56	0.005	S
S alb	4.00±0.35	3.54±0.42	<0.001	HS
PCR	894503±1248434	513988±671472	0.253	NS
IL6	0.70±0.52	1.31±0.65	<0.001	HS
Fibroscan	6.10±1.73	24.39±15.95	<0.001	HS
APRI	0.95±0.16	1.86±0.29	<0.001	HS
FIB4	1.34±0.28	3.9±0.31	<0.001	HS

Comparison between the two subgroups our results revealed that:

There was a significant decrease in the mean ± SD of WBCs in (Group Ib) (5.86 ± 2.41) when compared to (Group Ia) (7.40 ± 1.67) (P <0.05). And a highly significant decrease in the mean ± SD of each of platelets count and serum Albumin in (Group Ib) (126.22 ±56.90), (3.54 ± 0.42) when compared to (Group Ia) (233.14 ±54.92), (4.00 ± 0.35) respectively (P value < 0.001) (Table 2).

While There was a highly significant increase in the mean ± SD of IL-6, APRI and FIB-4 score and fibro scan in (Group Ib) (1.31±0.65), (1.86±0.29), (3.9±0.31) (24.39± 15.95) when compared to (Group Ia) (0.70 ±0.52) (0.95±0.16), (1.34 ± 0.28) (6.10 ±1.73) respectively (P value <.001) (Table 2). Also There was a significant increase in the mean ± SD of total serum bilirubin in (Group Ib) (1.15 ± 0.56) when compared to (Group Ia) (.73±0.33) (P <0.05) (Table 2).

Correlation of serum IL-6 with the studied parameters in group 1(40 patients with chronic HCV) showed that there was highly significant positive correlation between serum IL-6 and serum ALT, AST and Fibro scan (P value < 0.001) (Figure 2) and also significant positive correlation between serum IL-6 and serum bilirubin was noted (P value <0.05). While There was significant negative correlation between serum IL-6 and serum albumin (- 0.353) (P value <0.05) (Table 3).

Also there was a non significant negative correlation between serum IL-6 and WBCs count, platelets count, APRI score and FIB4 score (Table 3).

Table 3. Comparison between Correlation between IL6 concentration and studied parameters in all patient groups

Studied Parameters	Total patients Group I			Group Ia			Group Ib		
	IL 6		Sign.	IL6		Sign.	IL6		Sig.
	r value	P-value		r value	P-value		r value	P-value	
WBC (cell/cml)	-0.064	0.693	NS	0.335	0.128	NS	-0.344	0.162	NS
Hemoglobin concentration (gm/dL)	0.231	0.151	NS	0.154	0.434	NS	0.057	0.822	NS
RBC(cell/cml)	0.099	0.542	NS	0.132	0.545	NS	-0.177	0.483	NS
Platelet count (X109/L) (cell/cml)	-0.122	0.454	NS	-0.128	0.569	NS	-0.611	0.007*	S
ALT (IU/L)	0.711	<0.001**	HS	0.671	<0.001**	HS	0.762	<0.001**	HS
AST (IU/L)	0.576	<0.001**	HS	0.702	<0.001**	HS	0.592	0.010*	S
T Bilirubin (mg/dL)	0.339	0.032*	S	0.595	0.004*	S	0.510	0.031*	S
Serum Albumin (g/dL)	-0.353	0.002*	S	-0.328	0.136	NS	-0.488	0.040*	S
PCR (IU/ML)	0.242	0.178	NS	0.124	0.575	NS	0.323	0.095	NS
Fibroscan KPa	0.831	<0.001**	HS	0.568	0.006*	S	0.839	<0.001**	HS
APRI score	-0.175	0.280	NS	0.084	0.712	NS	0.266	0.286	NS
FIB4 score	-0.002	0.991	NS	0.088	0.698	NS	0.025	0.920	NS

In group 1a (20 patients with early fibrosis F0, F1 and F2) there was a significant positive correlation between serum IL-6 and each of serum bilirubin and fibro scan (P <0.05). Also, highly significant positive correlation between serum IL-6 and serum ALT, serum AST was noted (P <0.001).and non significant positive correlation between IL-6, PCR, APRI score and FIB4 score while serum IL-6 was non significant negatively correlated with platelets count and serum albumin (Table 3).

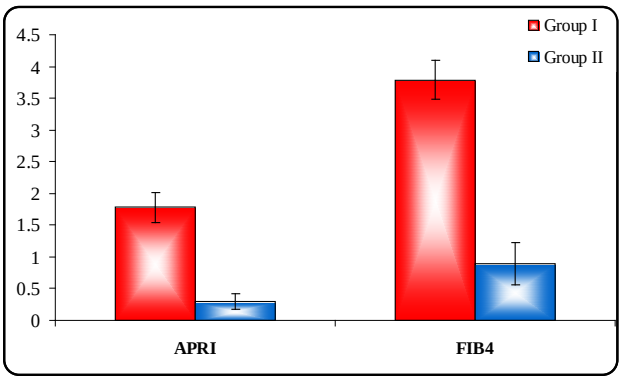

Figure 1. Comparison between (Group I) and (Group II) as regard APRI and FIB-4 score.

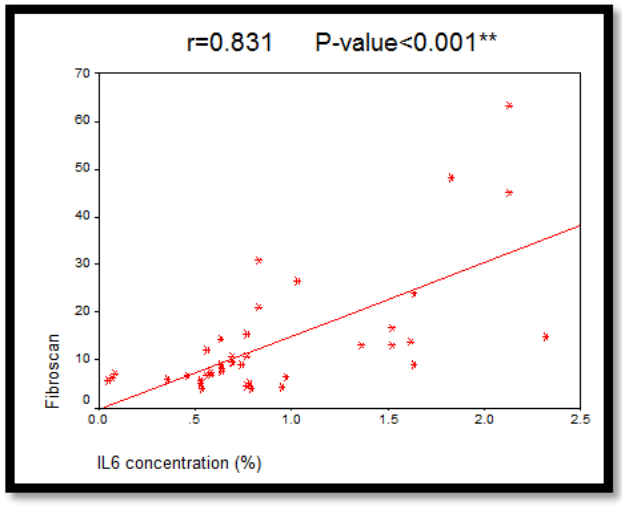

Figure 2. Correlation between serum IL-6 and fibroscan in all patients group (I)

Correlation of IL-6with the studied parameters in group 1b showed that there was a highly significant positive correlation between serum IL-6, ALT and fibro scan (P value <0.001) and also Serum IL-6 is significant positively correlated with AST and serum bilirubin, (P value <0.05).

While, serum IL-6 is significant negatively correlated with platelet count and serum albumin (P value <0.05). Also anon significant positive correlation between serum IL-6 and each of PCR, APRI score and FIB 4 score was noted (Table 3).

ROC curve of IL6 concentration was conducted for prediction of CLD within healthy control subjects. At IL6 concentration best cut off value of >=0.08, sensitivity was 100%, specificity was 92.5%; positive predictive value was 93% and negative predictive value was 100%.

4. Discussion

In our study, there is a highly significant elevation of serum level of IL6 in all patients with chronic HCV (group I) compared to control (group II) and in (group Ib) than in (group Ia) (P value <0.001).

This is in agreement with the study made by Abd El-Salam et al. (2017) [12] their study was conducted on 57 patients with chronic hepatitis C and 26 healthy people (the control group), they found a highly significant elevation of serum IL-6 in chronic HCV patients when compared to healthy control. Also in consistent with our results Nasr et al. (2016) [13] and Comanescu et al. (2015) [14], they reported that IL6 levels in patient group were high when compared to control group (p <0.001).

Another study which was done by Falasca et al. (2006), [5], who found that serum, IL-6 was significantly higher in patients with chronic HCV infection than healthy subjects.

On the other hand, Mourtzikou et al. (2014) [15], showed that there was no statistically significant difference in IL-6 level between Chronic HCV patients and controls. And Mohamed et al. (2015) [1] reported that in chronic HCV patients there was a non significant increase of serum level of IL-6 in patients group in comparison to healthy controls (P >0.05).

In our study, we found that there is no significant difference between levels of IL-6 in males patients when compared to female patients.

This is in agreement with Comanescu et al. (2015) [14] who found that higher levels of IL-6 were found in patients with HCV, without significant differences between both sex .This is in contrast to Nasr et al. (2016) [13] in their study who found that there is significant increase in serum levels of IL-6 in male patients when compared to females ($p<0.05$). These findings can be explained by; Estrogen has an inhibitory effect on IL-6 promoter activity by decreasing the activity of the transcription factors nuclear factor kB (NFkB).

Liver fibrosis is a reflection of the severity of liver damage in chronic hepatitis C. It is due to increased production and decreased destruction of the extracellular matrix [16]. Fibro Scan is a novel technology to diagnose liver fibrosis, which is based on the assessment of liver stiffness measurement (LSM) using ultrasound and low-frequency elastic waves [17].

It has been shown that fibro scan successfully detect fibrosis in chronic hepatitis C. Furthermore, it was reported that LSM was significantly increased with the stages of liver fibrosis in HCV patients [18].

In our study we found that fibro scan results in group Ib (atients with advanced fibrosis) was significantly high when compared with group Ia (patients with early fibrosis) ($P <0.001$).

agreement with our results Castera et al. (2005) [19] they found that fibro scan results was significantly increased in patients with advanced fibrosis than those with early fibrosis, and can be used as a non invasive method for assessment of liver fibrosis. Also in consistent with our study Elhariri et al. (2017) [20] who showed that fibro scan measurements were significantly increased in patients with progressive fibrosis in comparison with early fibrosis stages patients. They demonstrated that fibro scan is a reliable method in assessment of liver fibrosis in patients with chronic HCV in comparison to invasive technique (liver biopsy).

In our study there was no significant difference in HCV viral load measured by (real time PCR) in (group Ib) when compared with (group Ia) ($P >0.05$).

This is in agreement with Heller and Seeff (2005) [21], Pynard et al. (2001) [22] on their study on 2313 chronic HCV patients they stated that there was no significant association between viral load and progression of liver fibrosis. Also Javed et al. (2010) [23], stated in that changes in viral load was not associated with liver disease progression.

The mechanism of liver damage caused by HCV is not yet fully understood and could be attributed to either direct cytopathic effect of the virus or immune-mediated hepatic injury induced by HCV. Decline in serum HCV RNA levels in patients with chronic HCV infection can be explained by reducing number of hepatocytes and shrinking of liver mass with advancing of fibrosis [24].

On the other hand, Hisada et al. (2005) [25] on their study which made on HCV patients they reported that HCV RNA level is a predictor of liver fibrosis progression and ESLD death among persons with chronic HCV infection. Also another study done by Ijaz et al. (2011) [26] on 6048 patients with chronic HCV they found a relatively high viral load in initial fibrosis stages (F0-F1) (7.8 x 106±1.3 x 107) compared to advance fibrosis stages (F2-F3) (1.2 x 108±1.9 x 108) Also a significantly lower viral RNA levels in cirrhosis (F4) (2.9 x 105 ± 2.9 x 105).

In our study there was no significant correlation between IL-6 and PCR either in patients group (group I) or sub groups (group Ia and Ib) ($P >0.05$).

This is in agreement with Abd El-Salam et al. (2017) [12] who revealed that a non significant positive correlation between IL-6 and viral load.

In contrary to our study Zhang et al. (2011) [27], they reported that serum IL-6 has a significant negative correlation with HCV RNA viral load. And was significantly higher in genotype 1than in genotype 2 .Also our results disagree with Fanning et al. (1999) [28], who revealed that serum HCV RNA titer was significant positively correlated with degree of liver damage.

Our present study showed that there was a highly significant positive correlation between serum IL-6 and degree of liver fibrosis assessed by fibro scan in (groupI), (groupIb) ($P <0.001$). And a significant positive correlation of serum IL-6 and degree of liver fibrosis was found in (group Ia) ($P < 0.05$).

This is in agreement with Shah et al. (2015) [29] in the study made on (164 HIV-mono infected; 10 HCV-mono infected; 73 HIV/HCV-co infected; 59 neither infection) they showed that a positive correlation was significantly observed between serum IL-6 and liver fibrosis stage assessed by fibro scan in the HCV mono infected patients.

Also our results in consistent with Salter et al. (2013), [30], they reported that a significant positive correlation between serum IL-6 and liver fibrosis stage assessed by fibroscan

In our study there was a highly significant increase in APRI score and FIB-4 in (group I) when compared to (group II) and in (group Ib) when compared to (group Ia) ($p<0.001$).

This is in agreement with Motola et al. (2014) [31] and Lackner et al. (2005), [32], they demonstrated that APRI score and FIB4 was significantly increased in patients with advanced fibrosis and could be used as a strong non invasive predictors of fibrosis in chronic HCV patients.

Also our results agree with Macias et al. (2010) [33] who found that APRI score is significantly higher in patients with advanced liver fibrosis than in early fibrosis patients.

Another study done by Vallet et al. (2007) [10] found that FIB-4 is significantly increased in late fibrosis stages and FIB-4 can be used as in expensive and non invasive marker in assessing liver fibrosis in patients with chronic HCV which is in agreement with our study .

Our present study showed no significant correlation between IL-6 and APRI score and FIB-4 results in all studied groups (group I, Ia, Ib) ($P> 0.05$).

This is in contrast to study made by Fuster et al. (2013) [34] who reported that serum IL-6 was positively correlated with liver fibrosis assessed by FIB-4, which was done on HIV/ HCV patients with history of alcohol intake.

This can be explained by hypothesis that HIV/HCV co-infection could lead to an impaired cell-mediated immune response [35], and higher levels of inflammatory cytokines like IL-6, might be due to increased gut

permeability, bacterial translocation, CD4 cell depletion, and loss of Kupffer cells in the liver. These factors t have been related to fibro genesis in HIV/HCV co infection [36].

As regard liver functions tests in our study we found that, serum total bilirubin was significantly increased in patient (group I) when compared to control (group II), Also increased total serum bilirubin was significant in (group Ib), in comparison with (group Ia) ($p<0.05$).

These results were in agreement with Fahim et al. (2000) [37] who found that serum bilirubin was the highest in HCV infection with advanced stage of fibrosis .Also Ahmed et al. (2011) [38], proved a significant increase in total serum bilirubin in patient with late fibrosis stage in chronic HCV infected patients.

In consistent with our results Imbert-Bismut et al. (2001) [39] who found that serum bilirubin is elevated with progression of liver fibrosis and may be used as a serum marker to assess fibrosis stages and disease progression in chronic HCV patients.

As regard serum albumin our study revealed that it was significantly decreased in patients (group I) than control (group II) ($p<0.05$), while highly significant decreased in (group Ib) than (group Ia) ($p<0.001$).

This is in agreement with the finding of Fahim et al. (2000) [37] who found that serum albumin level showed significant decrease in HCV infected patient with advanced stage with fibrosis than those with early fibrosis.

In contrast to our study Wahib et al. (2005) [40] who founded that in the HCV-infected patients with late fibrosis, serum level of Albumin, were within the normal range.

In our study there was significant positive correlation between serum IL-6 and total billirubin in (group I, Ia and Ib) ($P < 0.05$).

In agreement with Mohmed et al. (2017) [2], on their study on 57 patients with chronic hepatitis C and 78 controls that showed positive correlation between IL-6 and serum total and direct bilirubin, this result may be due to the fact that increased direct billirubin is the result of decreased excretion from bile ducts due to viral hepatitis or cirrhosis.

Also, Genesca et al. (1999) [41] study on 66 patients with chronic HCV and 15 controls, reported that level of IL-6 is positively correlated with serum bilirubin and prothrombin time.

In contrast to our study Mourtzikou et al. (2014) [15] showed that serum IL6 has significant negative correlation with total billirubin.

In our study there was a significant negative correlation between serum IL-6 and serum albumin in (groupI & Ib) ($P < 0.05$).

Hypoalbuminemia is the result of the combined effects of. Inflammation and malnutrition in chronic liver disease Inflammation induces anorexia, reduces the effective use of dietary protein and energy intake, augments catabolism of albumin and decrease rate of synthesis in the liver [42].

This was in agreeing with Abd El-Ghafar et al. (2008) [43] who reported a significant negative correlation between serum IL-6 and albumin ($P < 0.05$).

In contrast to our results Abd El- Salam et al. (2017) [12] and Mohmed et al. (2017) [2] they found a significant positive correlation between IL-6 and albumin ($P < 0.05$).

As regard liver enzymes, our study showed that there is a highly significant increase in serum ALT & AST in (group I) when compared group (group II) ($P<0.001$), while there was no significant difference in ALT and AST (group Ib) when compared to (group Ia) ($P>0.05$).

This was in agreeing with Pouti et al. (1997) [44] who approved that, serum ALT levels have no significant increase in the presence of hepatic liver damage or fibrosis progression.

Bacon (2002) [45] reported that about 30% of patients with chronic HCV infection with advanced fibrosis reflect have normal serum ALT levels.

In contrast with Alberti et al. (2002) [46] who found that the severity of liver disease is accompanied by elevated ALT levels, and Boccato et al. (2006) [47] reported that serum ALT can be used as an extremely useful tool to asses disease progression in pt with hepatitis C.

Also In contrast to Wahib et al. (2005), [40], who reported that in the HCV-infected patients, the levels of ALT, AST were significantly increased in patients with advanced fibrosis.

Also Ahmed et al. (2011) [38], found a significant positive correlation between serum AST with fibrosis stage in chronic HCV infected patients. They reported that AST can be used as serum markers to assess the disease progression and fibrosis stages in chronic HCV patients.

Our study showed that there is highly significant positive correlation between IL6 and ALT in (group I & Ia and Ib) (P value <0.001).

This was in agreement with Mourtzikou et al. (2014) [15], who studied 40 patient with HCV infection and 30 control subjects and found a positive correlation between serum IL6 and ALT in patients with chronic HCV infection. This positive correlation between IL-6 and ALT suggesting that IL-6 level is closely related with liver inflammation status.

Also Zhang et al. (2011) [27] who studied 30 CHC patients and 30 healthy showing that serum IL-6 level was positively correlated with serum ALT in chronic HCV patients.

ALT is released by direct virus-related cytopathic activity and/or by an immune-mediated process [48].

IL-6 is one of the pro-and anti- inflammatory cytokines which are involved in the process of inflammation and tissue injury. In patients with chronic liver diseases, IL-6 expression in serum and liver tissue correlates with disease progression [49].

In contrast to Abd El-Salam et al. (2017), [12], who, revealed that there was a strong negative correlation between IL-6 and ALT which may indicate that the level of this marker (IL-6) may reflect liver injury despite low level of liver enzymes. As with liver damage, the damaged hepatocytes secrete fewer enzymes.

Also was in contrast with Haung et al. (1999) [50] who founded that the serum concentration of IL-6 was negatively correlated with parameters of hepatic inflammation (ALT, AST).

About AST our study showed that there is highly significant positive correlation of IL6 and AST in (group I& Ia) (P value <0.001), while there was only a significant positive correlation in (group Ib) (P value <0.05).

This goes in agreement with Abd El- Salam et al. (2017) [12] who reported a significant positive correlation between IL-6 and AST.

The increase in serum AST may be due to decreased clearance of AST from the affected sinusoidal cells [51].

In contrast to Mohamed et al. (2015), [1], who showed that there was a significantly negative correlation between IL-6 and AST (P >0.05) which may indicate that the level of this marker (IL-6) may reflect liver injury despite low level of liver enzymes.

Also in contrast with Abd EL-Ghafar et al. (2008) [43], Haung et al. (1999) [50] who reported a negative correlation between serum IL-6 with parameters of hepatic inflammation (ALT, AST).

5. Conclusion

The study concluded that serum level of IL-6 is significantly elevated in patients with chronic HCV infection and there was a highly significant positive correlation between IL-6 and stages of liver fibrosis assessed by fibroscan.

6. Recommendation

Serum level of IL-6 may be used as one of the non invasive markers in assessment of liver fibrosis and further studies should be done to assess trials for the addition of anti- IL-6 in the treatment of HCV patients to prevent the occurrence of complications.

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