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Biochemical Studies to Assess the Relationship of Interleukin-18 with Chronic Hepatitis C Virus Infection

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ABSTRACT: This study aimed to determine IL-18 and hs-CRP levels in patients at different stages of chronic HCV infection and to evaluate IL-18 as non invasive marker of the severity of liver damage in chronic hepatitis C patients. The present study was conducted on 70 Egyptian persons from Assuit governorate. Increased significant difference between serum levels of ALT and AST in chronic HCV patients than healthy controls. Also, increased insignificant differences between serum levels of ALP and bilirubin in chronic HCV patients than healthy controls. Regarding to PCR the present study showed insignificant positive correlation between serum IL-18 levels and results of PCR. The present study found insignificant positive correlation between serum IL-18 levels and liver function tests. Finally, the present study showed insignificant negative correlation between serum IL-18 levels and hs-CRP.

Keywords: HCV, IL-18, Assiut, hs-CRP

I. INTRODUCTION

Hepatitis C virus (HCV) is an enveloped RNA virus that belongs to the genus Hepacivirus within the family Flaviviridae [1]. HCV strains are classified into seven recognized genotypes (1-7) on the basis of phylogenetic and sequence analyses of whole viral genomes. Within each genotype, HCV is further classified into 67 confirmed and 20 provisional subtypes. Strains that belong to the same subtype differ at <15% of nucleotide sites [2].

HCV is a major cause of chronic hepatitis worldwide [3]. Approximately 70% of patients with chronic viremia develop chronic liver disease, 10-20% of which develop liver cirrhosis. Hundreds of thousands of people die each year from liver failure and liver cancer caused by this virus [4]. It is estimated that ~130 million people are persistently infected by HCV worldwide [5].

Egypt has higher rates of HCV than neighbouring countries as well as other countries in the world [6]. 14.7% of this population has been infected with this virus. Not everyone remains infected but Egyptian demographic health survey reported that 9.8% continue to have HCV RNA. That means almost 10% of the total population are infected and are infectious to other people. That is 7.8 million people with chronic active HCV infection [7].

Interleukins (ILs) are secreted proteins that bind to their specific receptors and play a role in the communication among leukocytes. The nomenclature is continuously evolving [8]. A major effect of IL-18 is the induction of cytokine synthesis. IL-18 induces IFN-Y production from T cells, and IL-13 from NK cells and T cells, especially in concert with other signals [9].

II. PATIENTS AND METHODS

A. Patients

Present study was carried out during the period from April to December 2014. It included 50 randomly patients proved serologically as having chronic viral hepatitis C. They were admitted to Assiut University Liver Hospital, and Dirout Fever hospital.

Patients suffering from hepatitis B virus infection or any other cause of viral liver cirrhosis were excluded. A brief history of each case was taken as regard to age, sex, and chronic diseases. Twenty healthy persons from Dirout Town, having normal liver enzymes and free from viral hepatitis markers were included as a control group.

B. Specimen collection

Ten ml of venous blood were collected aseptically from each person. Serum was separated by centrifugation and divided into two aliquots:

a. one aliquot was immediately used for estimation of liver function tests (ALT, AST, ALP and total, direct and indirect bilirubin) using test kits from Spin React Company for in vitro diagnostic use, and HCV antibodies by ELISA.

b. The other aliquot at -70 oC till sera were subjected to HCV-RNA detection by real time polymerase chain reaction (RT-PCR), ELISA technique for quantitative determination of IL-18 and immunofluorescence technique for quantitative determination of hs-CRP.

C. Methods

HCV antibodies by ELISA. AiD™ anti-HCV ELISA kit (Diagnostic Automation INC) employs solid phase, indirect ELISA method for detection of antibodies to HCV in two-step incubation procedure. Polyserne microwell strips are pre-coated with recombinant, highly immunoreactive antigens corresponding to the core and the non-structural regions of HCV (third generation HCV ELISA).

The amount of color intensity can be measured and it is proportional to the amount of antibody captured in the wells, and to the amount of antibody in the sample respectively. Wells containing samples negative for anti-HCV remain colorless [10].

Liver Function tests [11].

A- Alanine aminotransferase (ALT): Test was performed according to the manufacturer's instructions (spin React Company).

B- Aspartate aminotransferase (AST): Test was performed according to the manufacturer's instructions (spin React Company).

C- Alkaline phosphatase (ALP): Test was performed according to the manufacturer's instructions (spin React Company).

D- Total bilirubin (T. bil.): Test was performed according to the manufacturer's instructions (Diamond Company).

E- Direct bilirubin (D. bil.): Test was performed according to the manufacturer's instructions (Diamond Company).

F- Indirect bilirubin (Ind. bil.): Indirect bilirubin calculated by the difference between total and direct bilirubin.

Quantitative determination of HCV RNA by real time polymerase chain reaction (RT-PCR). RT-PCR was performed by QIAamp viral RNA Mini kit (QIAGEN) [12].

310 µl Buffer AVE added to the tube containing 310 µg lyophilized carrier RNA to obtain a solution of 1 µg/µl. The carrier RNA dissolved thoroughly; divide it into conveniently sized aliquots.

560 µl of prepared Buffer AVL containing carrier RNA pipetted into a 1.5 ml microcentrifuge tube.140 µl plasma, serum, urine, cell-culture supernatant, or cell-free body fluid added to the Buffer AVL–carrier RNA in the microcentrifuge tube. 3- 5 µl internal positive control (IPC) added into the mixture of lysis buffer and sample material. Plates incubated at room temperature (15–25°C) for 10 min. 560 µl of ethanol (96–100%) added to the sample, and mix by pulse-vortexing for 15 s. After mixing, briefly the tube centrifuged to remove drops from inside the lid. 650 µl of the solution from step 5 carefully applied to the QIAamp Mini column (in a 2 ml collection tube) without wetting the rim. Close the cap, and centrifuge at 8000 rpm for 1 min. The QIAamp Mini column placed into a clean 2 ml collection tube, and discard the tube containing the filtrate. The QIAamp Mini column carefully opened, and step 6 and 7 repeated.

The QIAamp Mini column carefully opened, and 500 µl of Buffer AW1 added. The cap closed, and centrifuged at 8000 rpm for 1 min. the QIAamp Mini column placed in a clean 2 ml collection tube and the tube containing the filtrate discarded. The QIAamp Mini column carefully opened, and 500 µl of Buffer AW2 added. The cap closed and centrifuged at full speed 14,000 rpm for 3 min.The QIAamp Mini column placed in a new 2 ml collection tube and the old collection tube with the filtrate discarded and centrifuged at full speed 14, 000 rpm for 10 min to dry the membrane completely. The QIAamp Mini column placed in a clean 1.5 ml microcentrifuge tube. The old collection tube containing the filtrate discarded. The QIAamp Mini column carefully opened and 60 µl of Buffer AVE added to the centre of the membrane. The QIAamp Mini column incubated at room temperature for 5 min and centrifuged at 8000 rpm for 1 min.

Quantitative determination of IL-18 by ELISA technique. Test kit was obtanied from Boster Biological Technology CO., Ltd 3942 B Valley Ave, CA, 94566, USA.

ELISA was performed according to the manufacturer's instructions. The Human IL-18 ELISA Kit measures human IL-18 by sandwich ELISA [13].

Quantitative determination of hs-CRP by immunofluorescence technique.

Principal:

Hs-CRP was performed by kit supplied by i-CHROMA TM hs-CRP Boditech Med Inc. lot No. 100513- S based on fluorescence immune assay technology. The i-CHROMA TM hs-CRP uses sandwich immune detection method by mixing the serum sample with detector buffer in a test vial, the fluorescence-labeled detector anti-CRP antibody in the buffer binds to CRP antigen on blood specimen [14]. As the sample loaded onto the sample well of the test device and migrates in nitrocellulose matrix of the test strip by capillary action , the complexes of detector antibody and CRP are captured to anti-CRP sandwich pair antibody that has been immobilizes on test strip. Thus the more CRP antigen on blood specimen the more complexes are accumulated on test strip. Signal intensity of fluorescence detector antibody reflects the amount of CRP captured is processed from i-CHROMA TM Reader.

D. Statistical analysis

Statistical presentation and analysis of the present study was conducted, using the mean, standard error, student t- test, Chi-square and Analysis of variance [ANOVA] tests by SPSS V17. All data were analyzed using the statistical package SPSS (version 12.0, SPSS Inc., Chicago, IL). Statistical analysis was performed using t-test, analysis of variance (ANOVA), 2- 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 0.05 was considered to be significant.

III. RESULTS

This study was conducted on 70 persons their ages ranged between 9-70 years. According to liver disease and response to treatment, patients were divided into the following subgroups:

1. Non Cirrhotic patients: They were 22 (84.61%) males and 4 (15.39%) female patients aged 49.5±10.595 (mean ± SD) years.
2. Cirrhotic patients: They were 19 (86.36%) males and 3 (13.64%) female patients aged 51.73±11.0332 (mean ± SD) years.
3. Hepatocellular Carcinoma (HCC) patients: They were 1 (50.0%) male and 1 (50.0%) female patients aged 60±5.656 (mean ± SD) years.
4. Non Hepatocellular Carcinoma (HCC) patients: They were 41 (85.41%) male and 7 (14.59%) female patients aged 50.52±10.740 (mean ± SD) years.

The mean of the age of patients were higher than those of controls with statistically significant (P= 0.003) (Table 1).

The highest distribution of the patients having normal liver (77.8%) at the age group from 29 to 48 years, while the highest distribution of the patients having cirrhotic liver (50%) and hepatocellular carcinoma (5.3%) at the age group from 49 to 70 years (Table 2 and Fig. 1).

Table 1: Means and standard deviation of the age of the studied Sample.

Variable	Studied sample groups		t-value	P-value
	Patients (N = 50)	Controls (N = 20)		
Age (years)	51.50 ± 9.74	38.65 ± 16.27	-3.302	0.003*

* Significant

Table 2: Distribution of the studied cases as regard to liver diseases in relation to age groups.

Age groups	Liver state							
	Normal liver (N = 26)		Cirrhotic (N = 22)		HCC (N = 2)		Total	
	N	%	N	%	N	%	N	%
9-28 (ys)	2	66.7	1	33.3	0	0.0	3	100
29-48 (ys)	7	77.8	2	22.2	0	0.0	9	100
49-70 (ys)	17	44.7	19	50.0	2	5.3	38	100

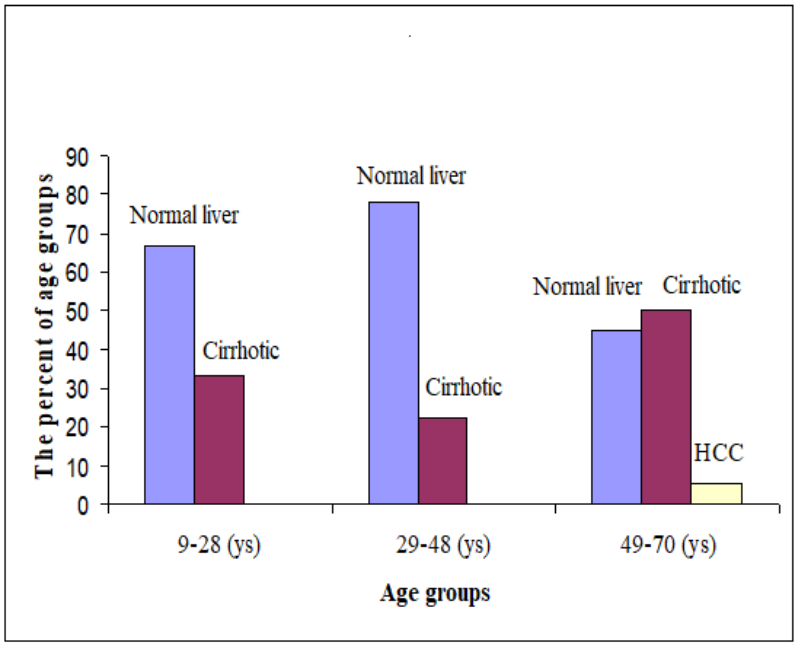

Fig. 1. Distribution of the studied cases as regard liver diseases in relation to age groups.

Our result showed that the highest distribution of the male patients were having normal liver (52.4%) than the distribution of the male patients having hepatocellular carcinoma (2.4%) and the highest distribution of the female patients having normal liver (50%) than the distribution of the female patients having hepatocellular carcinoma (12.5%). Also, the highest distribution of male cirrhotic cases (45.2%) than the distribution of female cirrhotic cases (37.5%) was recorded (Table 3 and Fig. 2).

Our results showed that statistical significant difference between Hepatitis C patients and Control as regards to the mean values of [ALT (P = 0.002), AST (P = 0.005)] and high statistically significant difference between Hepatitis C patients and Control as regards the mean values of [Ind.bil (P = 0.000)] (Table 4). However there was no significant difference was observed as regard to the mean value of [ALP (P = 0.08), T.bil (P = 0.01) and D.bil (P = 0.01)] (Table 4).

Table 3: Distribution of the studied cases as regard to liver diseases in relation to gender.

Gender	Liver state							
	Normal liver (N = 26)		Cirrhotic (N = 22)		HCC (N = 2)		Total	
	N	%	N	%	N	%	N	%
Male	22	52.4	19	45.2	1	2.4	42	100
Female	4	50.0	3	37.5	1	12.5	8	100

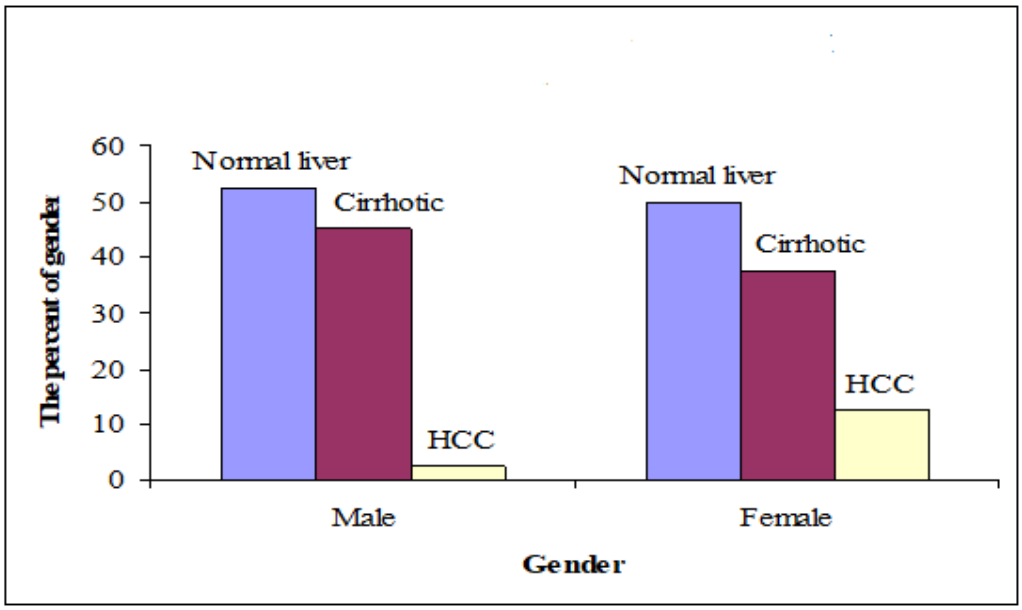

Fig. 2. Distribution of the studied cases as regard liver diseases in relation to gender.

Table 4: Comparison between the studied groups and controls regard to the mean values of liver function tests.

Variables	Studied groups		t-value	P-value
	Patients (N = 50)	Controls (N = 20)		
ALT (< 40 U/L)	49.78±34.63	28.00 ±25.003	-2.93	0.002*
AST (< 37 U/L)	58.16 ±35.69	33.10 ±35.564	-2.66	0.005*
ALP (up to 240 U/L)	191.72±89.161	145.70±136.159	-1.396	0.08
Total Bilirubin (0.2-1.2 mg/dl)	1.344±1.604	0.810 ±0.165	-2.306	0.01
Direct Bilirubin] (up to 0.3 mg/dl)	0.39±0.517	0.200±0.056	-2.549	0.01
Indirect Bilirubin (up to 0.9 mg/dl)	0.954 ±1.09	0.61±0.145	-9.725	0.000**

* Significant ** High Significant

Present study showed that no statistical significant difference between cirrhotic and non cirrhotic patients as regard to the mean values of [ALT (P = 0.5), AST (P = 0.24), ALP (P = 0.29), Total bilirubin (P = 0.19), Direct bilirubin (P = 0.14) and Indirect bilirubin (P = 0.15)] (Table 5).

Table 5: The Mean values of liver function tests among Cirrhotic and Non cirrhotic chronic Hepatitis C patients.

Variables	Patients infected with HCV		t-value	P-value
	Cirrhotic cases (N = 22)	Non-cirrhotic cases (N =28)		
ALT (< 40 U/L)	49.73 ±28.422	49.82 ±39.351	0.009	0.5
AST (< 37 U/L)	62.05 ±34.150	55.11 ±37.185	-0.692	0.24
ALP(up to 240 U/L)	199.86±105.64	185.32±75.167	-0.546	0.29
Total Bilirubin (0.2-1.2 mg/dl)	1.695±2.364	1.068±0.374	-0.874	0.19
Direct Bilirubin (up to 0.3 mg/dl)	0.491±0.753	0.311±0.173	-1.09	0.14
Indirect Bilirubin (up to 0.9 mg/dl)	1.205 ±1.619	0.757 ±0.249	-1.286	0.15

Our study showed that the means of liver function tests levels were higher among the hepatocellular carcinoma patients [ALT, AST, ALP, Total bilirubin, Direct bilirubin and Indirect bilirubin] than among the non hepatocellular carcinoma patients with no statistically significant. [ALT (P = 0.13), AST (P = 0.07), ALP (P = 0.02), Total bilirubin (P = 0.9), Direct bilirubin (P = 0.848) and Indirect bilirubin (P = 0.9)] (Table 6). Present study showed that the insignificant negative correlation between ALT, AST and ALP with PCR results [ALT (P = 0.639), AST (P = 0.391) and ALP (P = 0.421)], and the insignificant Positive correlation between IL-18 and hs-CRP with PCR results [IL-18 (P = 0.297) and hs-CRP (P = 0.287)] (Table 7). Our study showed that the insignificant positive correlation between IL-18 and hs-CRP with age [IL-18 (P = 0.38) and hs-CRP (P = 0.20)] (Table 8).

Table 6: The Mean values of liver function tests among Hepatocellular carcinoma (HCC) chronic Hepatitis C Patients and Non Hepatocellular carcinoma patients.

Variables	Patients infected with HCV		t-value	P-value
	Patients having HCC (N = 2)	Patients having no HCC (N = 48)		
ALT (< 40 U/L)	80.00±55.154	48.52 ±33.843	-0.801	0.13
AST (< 37 U/L)	118.00±76.368	55.67 ±32.330	-1.15	0.07
ALP (up to 240 U/L)	330.00±113.137	185.96±84.664	-1.78	0.02
Total Bilirubin (0.2-1.2 mg/dl)	0.900±0.283	1.363 ±1.634	1.489	0.9
Direct Bilirubin (up to 0.3 mg/dl)	0.250±0.071	0.396±0.527	1.09	0.84
Indirect Bilirubin (up to 0.9 mg/dl)	0.650 ±0.212	0.967±1.12	1.505	0.9

Table 7: Correlation between IL-18, Hs-CRP and liver function tests with PCR results of the studied cases.

Correlations	r	p
PCR and ALT	-0.057	0.639
PCR and AST	-0.104	0.391
PCR and ALP	-0.098	0.421
PCR and IL-18	0.126	0.297
PCR and Hs-CRP	0.124	0.287

Table 8: Correlation between IL-18 and Hs-CRP with the age of the studied cases.

Correlations	r	p
IL-18 and age	0.105	0.38
Hs-CRP and age	0.15	0.20

Present study showed that the insignificant positive correlation between ALT, AST and ALP with IL-18 levels [ALT (P = 0.07), AST (P = 0.81) and ALP (P = 0.089)]. And the insignificant negative correlation between ALT, AST and ALP with hs-CRP levels [ALT (P = 0.81), AST (P = 0.785) and ALP (P = 0.234)] (Table 9).

Table 9: Correlation between IL-18 and Hs-CRP with liver function tests of the studied cases.

Correlations	r	p
IL-18 and ALT	0.21	0.07
IL-18 and AST	0.29	0.81
IL-18 and ALP	0.204	0.089
Hs-CRP and ALT	0.029	0.81
Hs-CRP and AST	-0.033	0.785
Hs-CRP and ALP	-0.144	0.234

Our data recorded that the insignificant negative correlation between IL-18 and hs-CRP (P = 0.613) (Table 10).

Table 10: Correlation between IL-18 and Hs-CRP of the studied cases.

Correlations	r	p
Hs-CRP and IL-18	-0.061	0.613

IV. DISCUSSION

Egypt has a high prevalence of HCV and a high morbidity and mortality from chronic liver disease, cirrhosis and hepatocellular carcinoma. The most prevalent genotype in Egypt is 4a [15]. HCV-4 is considered a major cause of chronic hepatitis, cirrhosis, hepatocellular carcinoma and liver transplantation in Africa and Middle East. Although HCV-4 is the cause of approximalty 20% of the 170 million cases of chronic hepatitis C in the world, it has not been the subject of widespread research [6].

Cytokines are recognized as an important factor in the pathophysiology of chronic hepatitis C. Hs-CRP plays a major role in the scenario of an activated systemic inflammatory response, and is also a common feature of various chronic liver diseases, such as nonalcoholic fatty liver disease and nonalcoholic steatohepatitis [16].

The present study was conducted on 70 subjects divided into 2 groups: Group I which included 50 patients with chronic hepatitis C infection and group II (controls) which included 20 apparent healthy subjects. Among 50 patients were 42 (84.0%) males and 8 (16.0%) females patients aged 51.50±9.74 (mean ± SD) years with a range of 21 to 67 years.

The distribution of the studied patients were higher at age group from 49 to 70 years (84.4%) than the distribution of controls (15.6%). This findings agreed with Bello *et al.,* (2002), who founded that a significant correlation between age at the time of infection and a more advanced stage of the disease (P=0.001) [17]. Also, these results were in agreement with Poynard *et al.,* (2001), who reported that age at the time of infection was found to be one of the most important host-related factors in the progression of liver fibrosis, as also observed in other studies [18].

The distribution of patients having cirrhotic liver (50%) were higher than the distribution of the patients having hepatocellular carcinoma (5.3%) at the age group from 49 to 70 years. Also, the distribution of the cases having normal liver (77.8%) was higher than the distribution of the cases having cirrhotic liver (22.2%) at the age group from 29 to 48 years. This study agreed with Reham (2012), reported a significant correlation between age and stage of fibrosis as patients with stages 3 and 4 hepatic fibrosis had significantly higher mean age than patients with other stages of fibrosis (P = 0.01) [19]. Also, these findings were in agreement with Bello *et al.,* 2002, who founded that patients who were infected after 40 years of age had a higher rate of fibrosis progression (P<0.001) [17].

Our results showed that the distribution of male cirrhotic cases (45.2%) higher than the distribution of female cirrhotic cases (37.5%). These results agreed with Marcellin and Boyer (2002), who reported that Older age, male gender, excessive alcohol consumption, overweight, and immune deficiency are associated with more rapid progression of fibrosis [20].

In our various laboratory and clinical parameters were analyzed when compared between chronic HCV patients and healthy controls, a highly significant increased in patients with chronic HCV infection and healthy controls in their AST levels (p =0.005), ALT levels (p=0.002) and indirect bilirubin levels (p=0.000). Also, the present study showed that, a non significant increased in patients with chronic HCV infection and healthy controls in their ALP levels (p =0.08), total bilirubin levels (p=0.01) and direct bilirubin levels (p=0.01). There were in agreement with Persico *et al.* (2000) showed that the patients had an increase in serum ALT levels with no significant progression of histological liver lesions [21].

Also, the serum ALT level is recognized as a marker reflecting the degree of the histological damage and has served as a parameter for starting therapy or judging response to antiviral treatment in chronic hepatitis C [22].

However the various laboratory and clinical parameters were analyzed when compared between chronic HCV patients with cirrhotic liver and chronic HCV patients without cirrhotic liver; a non significant increased in patients with cirrhotic liver than in patients without cirrhotic liver in their AST levels (p =0.24), ALT levels (p=0.5) and ALP levels (p =0.29), total bilirubin levels (p=0.19), direct bilirubin levels (p=0.14) and indirect bilirubin levels (p=0.15). This study was in agreement with studies of Ahmed *et al.,* (2009) who found that elevation in serum ALT and AST levels by increasing the scores of the liver biopsy grades and liver biopsy stages; however these results were not statistically significant [23].

The present study data confirmed hypothesis that the cellular immune response in patients of HCV infection with persistent normal ALT levels is less activated than in patients with abnormal ALT levels hepatocyte apoptosis has an important role since chronic liver damage and hepatocyte cell loss by apoptosis could occur in HCV-infected patients without overt ALT level changes, explaining the progressive nature of liver disease that was presented in patients with a normal ALT level [24].

Also, we investigated the various laboratory and clinical parameters were analyzed when compared between chronic HCV patients with hepatocellular carcinoma (HCC) and chronic HCV patients without hepatocellular carcinoma; the present study showed that, a non-significant increased in patients with hepatocellular carcinoma than in patients without hepatocellular carcinoma in their AST levels (p =0.07), ALT levels (p=0.13) and ALP levels (p =0.02), total bilirubin levels (p=0.9), direct bilirubin levels (p=0.84) and indirect bilirubin levels (p=0.9). In general, chronic hepatitis C patients with elevated ALT levels and high HCV-RNA titers in the sera are considered to have active HCV replication in the liver and to be at risk for continued liver injury [22].

We recorded that regarding to PCR the present study showed insignificant positive correlation between serum IL-18 levels and results of PCR (r = 0.126, P=0.297). These findings were in agreement with EL-Sherif *et al*., (2013), who found that non-significant positive correlation between serum IL-18 and results of PCR in group II (HCV patients with elevated liver enzymes) [25].

Present study showed that there was insignificant positive correlation between serum IL-18 levels and ALT, AST and ALP [ALT (r = 0.159, P=0.18), AST (r = 0.21, P=0.07) and ALP (r = 0.204, P=0.089)]. These findings were in agreement with Sharma *et al.,* (2009), who reported that concentrations of IL-18 were not associated, neither with age nor with ALT levels [26]. On the other side, these findings were in disagreement with Nadia *et al*., (2013), who found a significant moderate correlation between IL-18 of HCV pa¬tients and their AST levels (r =0.3, p =0.04) [27].

These conflicts may be explained; in the present study HCV patients who had elevated liver enzymes were small group but the others who had normal liver enzymes were the most patients.

Finally, our results showed that insignificant negative correlation between serum IL-18 levels and hs-CRP (r = -0.061, p = 0.613). These findings were in disagreement with Ludwiczek *et al.,* (2002), who found the correlation of IL-18 with C-reactive protein as a marker of inflammation and with GOT as a marker of hepatocyte damage [28].

V. CONCLUSION

This study aimed to evaluation of the relationship of IL-18 and hs-CRP in chronic HCV infection. IL-18 is a non-invasive marker of the severity of liver damage in chronic hepatitis C patients. IL-18 is an important factor for the evaluation of HCV treatment by interferon regimens. Hs-CRP is a confirmed marker of the severity of liver damage in chronic hepatitis C patients.

RECOMMENDATIONS

1. Further studies using a large numbers of Egyptian patients with HCV-related chronic liver disease are required to confirm the association of IL-18 with the response to interferon therapy in HCV.
2. These investigations could help to identify patients with markedly increased risks of disease progression and could guide the design of individualized treatment strategies for chronic hepatitis C.

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Authors' Contribution

All authors are in agreement with the content of the manuscript and were involved in all steps of its preparation.

Conflict of interest statement. None of the authors has an affiliation or conflict of in terest.

REFERENCES

[1]. Moradpour, D., Penin, F. and Rice, C.M., (2007). Replication of hepatitis C virus. *Nat Rev Microbiol,* **5**, 453–463.

[2]. Smith, D.B., Bukh, J., Kuiken, C., Muerhoff, A.S., Rice, C.M. and Stapleton, J.T., (2014). Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: updated criteria and genotype assignment Web resource. *Hepatol,* **59**: 318–327.

[3]. World Health Organization. Hepatitis C. World Health Organization; 2019. Available from: https://www.who.int/news-room/fact-sheets/detail/hepatitis-c.

[4]. Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R.L., Torre, L.A. and Jemal, A., (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CACancer J. Clin, vol. **68**, 394–424.

[5]. Yang, J.D., Hainaut, P., Gores, G.J., Amadou, A., Plymoth, A. and Roberts, L.R., (2019). A global view of hepatocellular carcinoma: Trends, risk, prevention

and management. *Nat. Rev. Gastroenterol. Hepatol*, **16**, 589–604.
[6]. Khattab, M.A., Ferenci, P., Hadziyannis, S.J., Colombo, M. and Manns M.P., (2011). Management of hepatitis C virus genotype 4: recommendations of an international expert panel. *Journal of Hepatol,* **54**: 1250-62.
[7]. El-Zanaty, F., El-Zeini, L.O., Way, A. and Casterline J.B., (2009). Egypt Demographic and Health Survey 2008. Cairo, Egypt: Ministry of Health, El-Zanaty and Associates, and Macro International. *Int Fam Plan Perspect*, **29**: 158-66.
[8]. Dinarello C., Arend W., Sims J., Smith D. and Blumberg H. (2010). IL-1 family nomenclature. *Nat. Immunol.,* **11**: 973.
[9]. Yang J., Zhu H., Murphy T.L., Ouyang W. and Murphy K.M. (2001). IL-18-stimulated GADD45b required in cytokine-induced, but not TcR-induced IFN-Y production. *Nat. Immunol*. **2**: 157–164**.**
[10]. Santos, V.C.D., Schinoni, M.I., Oliveira, I.S., Atta, M.L.S., Atta, A.M., (2019). IgG1 and IgG4 antibodies against Core and NS3 antigens of hepatitis C virus. *Rev Soc Bras Med Trop,* **52**: e20180491.
[11]. Titez, N.W., (1995). Clinical guide to Laboratory tests. 3rd ed. Philadephia WB Saundars; 268-273.
[12]. Xiaowei, W. and Brian, S., (2003). A PCR primer bank for quantitative gene expression analysis. *Nucleic Acids Res.*, **31**(24): e154; 1-8.
[13]. Okamura, H., Nagata, K., Komatsu, T., Tanimoto, T., Nukata, Y., Tanabe, F., Akita, K., Torigoe, K., Okura, T., Fukuda, S. and Kurimoto, M., (1995): A novel costimulatory factor for gamma interferon induction found in the livers of mice causes endotoxic shock. *Infect. Immun.,* **63**, 3966–3972.
[14]. Del Giudice, M. and Gangestad, S.W., (2018). Rethinking IL-6 and CRP: Why they are more than inflammatory biomarkers, and why it matters. *Brain Behav. Immun.,* **70**, 61–75.
[15]. Faisal, N., Sidra, S.H., Ghulam, M. and Shumaila, N., (2015). An overview on hepatitis C virus genotypes and its control. Egyptian Journal of Medical Human Genetics, **16**(4): 291–298.
[16]. Abaete De los Santos, C., Antonello, V. and Antonello, I., (2017). An Assay: Hepatitis C does not incite increased C-reactive protein level in hemodialysis patients unless another inflammatory condition is associated. *J. Gastroenterol. Hepatol. Res.*, **6**, 2416–2418.
[17]. Fabregat, I. and Caballero-Diaz, D., (2018). Transforming Growth Factor-beta-Induced Cell Plasticity in Liver Fibrosis and Hepatocarcinogenesis. *Front. Oncol.,* **8**, 357.
[18]. Poynard, T., Ratziu, V. and Charlotte, F., (2001). Rates and risk factors of liver fibrosis progression in patients with chronic hepatitis C. *J. Hepatol,* **34**(5): 730-9.
[19]. Reham, A.S. (2012). Correlation between alanine aminotransferase level, HCV-RNA titer and fibrosis stage in chronic HCV genotype 4 infection. *The Egyptian Journal of Medical Human Genetics*, **13**: 207–212.
[20]. Xiang, D. M., Sun,W., Ning, B.F., Zhou, T.F., Li, X.F., Zhong, W., Cheng, Z., Xia, M.Y., Wang, X., Deng, X., (2018). The HLF/IL-6/STAT3 feedforward circuit drives hepatic stellate cell activation to promote liver fibrosis. Gut, **67**, 1704–1715.
[21]. Sarrazin, C., Isakov, V., Svarovskaia, E.S., Hedskog, C., Martin, R., Chodavarapu, K., (2017). Late relapse versus hepatitis C virus reinfection in patients with sustained virologic response after sofosbuvir-based therapies. *Clin Infect Dis,* **64**: 44-52.
[22]. Elsayed, D., Noby, M.A., Mostafa, A.H., El-Din, M.G. and El Kadera, E.O., (2018). Low cost detection of hepatitis C virus RNA in HCV infected patients by SYBR Green I real-time PCR. *Alexandria Journal of Medicine,* **54**(4): 481-485.
[23]. Ahmed, N., Holcroft, C., Szilagyi, A., Lamoureux, E. and Hilzenrat N., (2009). No association between ALT levels and liver biopsy results in patients evaluated for undetermined liver enzymes elevation. *Can J Gastroenterol,* **23**: 62-8.
[24]. Marrero, J.A., Kulik L.M., Sirlin, C.B., Zhu A.X., Finn R.S. and Abecassis M.M., (2018). Diagnosis, staging, and management of hepatocellular carcinoma: 2018 practice guidance by the American Association for the Study of Liver Diseases. *Hepatol*, **68**: 723-750.
[25]. EL-Sherif A.S., Kamel H.M., Aml M.K. and Faten M.K., (2013). Serum Interluekin-18 in Hepatitis C Virus Infection. *MJM*, **24**(1): 1- 6.
[26]. Sharma, A., Chakraborti, A., Das, A., Dhiman, R.K. and Chawla, Y., (2009). Elevation of interleukin-18 in chronic hepatitis C: implications for hepatitis C virus pathogenesis. *Immunol,* **128**: e514–e522.
[27]. Nadia, A.A., Faten, Z.M., Mohamed, S.E.l. and Manal S.F., (2013): A study of serum interleukin-18 level in chronic hepatitis C infected patients in Suez Canal Area. *Indian Journal of Applied Research*, **3**(9): 28-30.
[28]. Serti, E., Werner, J.M., Chattergoon, M., Cox, A.L., Lohmann, V. and Rehermann, B., (2014). Monocytes activate natural killer cells via inflammasome-induced interleukin 18 in response to hepatitis C virus replication. *Gastroenterology*, **147**, 209–220.