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The Role of Interleukin-18 in Egyptian Patients Infected with Chronic Hepatitis C Virus and Treated with Sofosbuvir

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Abstract

IL-18 belongs to the IL-1 family. According to recent studies, IL-18 may contribute to the aetiology of liver damage in both acute and chronic hepatitis, as well as the beginning and progression of liver fibrosis. The goal of this study is to determine how interleukin 18 influences how effectively individuals with hepatitis and hepatitis C in Egypt respond to Sofosbuvir therapy. The current study included 400 individuals with HCV, who were recruited at random. Every sample was acquired from Egypt's Sohag Hospital, and the trial ran from January 1, 2020 to January 30, 2022. To identify HCV RNA, all samples were tested with reverse transcriptase polymerase chain reaction (RT-PCR), and anti-HCV antibodies were detected using the ELIZA approach. All patients were treated with Sofosbuvir. According to our findings, 354 (88.5%) of the 400 people who received sofosbuvir for Hepatitis C had treatment-related side effects, whereas just 46 (11.5%) did not. We randomly selected 80 individuals, 40 of whom responded positively to pharmaceutical therapy and 40 who did not. Blood samples were taken during routine clinic visits for various tests.We looked at two patient populations: those who responded to sofosbuvir treatment (n = 40) and those who did not respond (n = 40). (patients with positive HCV RNA and anti-HCV antibodies). Our study found that those who respond to sofosbuvir treatment had lower levels of interleukin-18 after treatment, but those who do not receive sofosbuvir treatment have greater levels of interleukin 18. Hepatitis C Virus.

Keywords: Hepatitis C Virus, Sofosbuvir, Treatment, Side effect, Complication. interleukin-18

1. Introduction

Over 170 million people worldwide are infected with the hepatitis C virus (HCV), a severe health hazard [1]. In Egypt, 7.2 million people, or over 12% of the population, have it [2]. About 85% of

infected people are predisposed to chronic HCV infection, which raises the risk of tragic outcomes such as liver cirrhosis and hepatocellular carcinoma (HCC) [3]. In response to chronic infection, circulating monocytes and tissue macrophages, including Kupffer cells of the liver, become activated and release a variety of cytokines, including proinflammatory ones [4]. The most serious public health issue Egypt faces is hepatitis C virus (HCV), which has the world's highest prevalence rate. According to research, 9.8% of Egyptians are actively infected with HCV, and 14.7% have HCV antibodies [5, 6]. Prevalence rates in particular age groups may reach 50%. The regional distribution of anti-HCV in individuals aged 10 to 50 is 28% in the Nile Delta and 26% in Upper Egypt [7]. A trial with sofosbuvir and ribavirin, a DAA combination, demonstrated pan genotypic clinical efficacy in HCV genotypes 1-6 [8]. Since then, the Egyptian Ministry of Health's proposal for a new national plan to limit the HCV pandemic in Egypt, which requests increased capital investment, has garnered support from the WHO and other organizations [9]. The major treatment indicated by this strategy, named "The Plan of Action for the Prevention, Care, and Treatment of Viral Hepatitis 2014-2018," is sofosbuvir (SofosbuvirTM, Gilead Sciences, San Francisco, USA) [10]. Interlukin-18 (IL-18) is a pleiotropic pro-inflammatory cytokine formerly known as interferon-gamma-inducing factor. It is predominantly generated by macrophages and peripheral blood mononuclear cells. Chronic hepatitis C virus infection is associated with considerable up-regulation of IL-18 in the inflammatory infiltrate, indicating that this cytokine may play a role in the disease's ongoing cellular immunological response against hepatocytes [8]. It is well understood that viral infections inhibit IL-18 and increase IL-18 binding protein, both of which impair the immune system [11]. Because HCV is difficult to eradicate, around 20% of patients with chronic infection develop cirrhosis with an increased risk of hepatocellular carcinoma [12]. Hs-CRP, or high-sensitivity C-reactive protein, is a non-specific inflammatory marker. Similar to body temperature, it is effective in therapeutic settings but lacks specificity in identifying a specific illness [13]. Hs-CRP is a trait common to a number of chronic liver illnesses, including nonalcoholic steatohepatitis and nonalcoholic fatty liver disease, and it is crucial when the systemic inflammatory response is activated [14,15]. This study sets out to investigate serum IL-18 levels in liver disorders associated with chronic HCV and responded to treatment with Sofosbuvir drug.

2. Patients and Methods

2.1 Collection and processing of blood samples

The Sohag Hospital in Egypt provided all the samples, and the study took place between January 1, 2022, and January 30, 2023. A total of 400 HCV patients were tested for HCV RNA using the reverse transcriptase polymerase chain reaction (RT-PCR) and anti-HCV antibodies using the ELIZA technique. Every patient was treated with Sofosbuvir. Our findings demonstrate that of the 400 patients treated with Sofosbuvir for hepatitis C, 354 (88.5%) had treatment-related side effects, whereas just 46 (11.5%) did not. Eighty patients were picked at random; forty responded positively to medicine, whereas forty did not. During routine clinic appointments, blood samples were collected for a variety of tests. Our study included two patient groups: those with anti-HCV positive and HCV RNA negative status who responded to Sofosbuvir treatment (n = 25) and those who did not respond (n = 25). (patients with positive HCV RNA and anti-HCV antibodies).

2.2 Serum Markers for HCV Infection

Antibody detection: Enzyme-linked immunosorbent tests (ELIZAs; BioKit-bioelisa HCV 4.0) of the third generation were used to detect anti-HCV antibodies.

Recombinant antigens that represented the main NS3, NS4, and NS5 epitopes of HCV were coated in the microplate wells. The concentration of HCV antibodies in the sample is correlated with color intensity. (lot: BI25392).

2.3 Hematological assessment:

This was done by complete blood count (CBC) was determined using an automated hematology analyzer DIRUI BCC-3000B.

2.4 Analyses biochemical (MICR LAB RX-199 Bio-Chemistry Analyzer) Colorimetric technique for aspartate aminotransferase (AST/SGOT)

The reversible transfer of an amino group from aspartate to alpha-ketoglutarate, which forms glutamate and oxaloacetate, is catalyzed by aspartate aminotransferase (AST), formerly known as glutamate oxaloacetate (GOT). The resulting oxaloacetate is degraded to NADH and malate dehydrogenase (MDH). Photometric measurements of the rate of decrease in NADH concentration show a positive correlation with the catalytic concentration of AST in the sample. (GOTS0205023)

2.5 Colorimetric technique for Alanine aminotransferase (ALT/SGPT)

Glutamate pyruvate transaminase (GPT), also known as alanine aminotransferase (ALT), catalyzes the reversible transfer of an amino group from alanine to alpha-ketoglutarate, resulting in the formation of glutamate and pyruvate. With the help of lactate dehydrogenase (LDH) and NADH, the generated pyruvate is converted to lactate. The photometrically observed rate of decline in NADH concentration is directly proportional to the catalytic concentration of ALT in the sample. (LOT:GPTS0109020).

2.6 Total bilirubin

The total bilirubin concentration is determined in presence of caffeine by the reaction with diazotized acid to produce an intensely colored diazo dye. The intensity of color of this dye is proportional to the concentration of total bilirubin. (LOT: BILI0107020-2).

2.7 Test of Inflammatory Index for C-reactive protein (CRP)

The IchromaTM CRP Kit (LOT: CRQBK06) uses a sandwich immuno-detection technique in which the blood specimen in the test vial is mixed with the detection buffer. This causes the detector anti-CRP antibody in the buffer, which is fluorescently labeled, to attach to the CRP antigen in the blood specimen. The sample combination is loaded and migrates across the test cartridge's matrix, capturing the complexes between the detection antibody and CRP on the immobilized anti-CRP sandwich pair antibody. A pre-programmed calibration procedure converts the fluorescence intensity into CRP concentration. The reader is presented with the test results as ng/mL for CRP. (I Chroma device, type I Chroma PCT, was used to read the reaction).

2.8 Quantitative measurement of IL-18 using the ELIZA method:

All research subjects' sera were tested for IL-18 levels using a commercially available Enzyme linked Immunosorbent Assay Kit (Boster Biological Technology CO., Ltd., 3942 B Valley Ave., CA, 94566, USA), in accordance with the manufacturer's instructions. In a nutshell, the assay employs two monoclonal antibodies directed against two distinct human IL-18 epitopes. The anti-human IL-18 monoclonal antibody-coated wells were used to incubate standards or samples for measurement. An anti-human IL-18 monoclonal antibody conjugated with peroxidase was applied to the microwell

and incubated after washing. The chromogen and peroxidase substrate were combined and left to incubate for a further period of time following another washing. The enzyme reaction was then stopped, and the generated color was stabilized by adding an acid solution to each well. Next, using a microplate reader, the optical density (OD) of each well was determined at 450 nm. Using reference standards as a base, a dosage response curve was used to calibrate the serum IL-18 levels. (LOT: A07261)

2.9 Molecular assay

2.9.1 Separating RNA and RT-PCR (DT Prime Real-time Detection Thermal Cycler)

The Amplicor technique (Basel, Switzerland) of qualitative polymerase chain reaction (PCR) was used to test for the viral genome in serum. Viral RNA was isolated using the viral RNA extraction kit from Qiagen in Hilden, Germany, in accordance with the manufacturer's instructions. Complementary DNA (cDNA) of the first strand was created. Initial denaturation was placed for five minutes at 95 °C. For a total of 40 cycles of polymerase chain reaction amplification, the following temperatures were used: 94°C for 1 min, 57 ° C (annealing temperature) for 1 min, 72° C for 1 min, and 72° C for the final extension of 7 min. The forward primer was used with the pattern 5 ′ CGCGCGACTAGGAAGACTTC3 ′ , while the reverse primer was used with the sequence 5 ′ ACCCTCGTTTCCGTACAGAG 3′ .

2.9.2 Detection Using Agarose Gel Electrophoresis

The PCR-HCV products were found using electrophoresis on 1.5% agarose gel, stained with ethidium bromide, and examined under UV light responses.

2.9.3 PCR-based HCV genotyping

Only ten samples from various chronic HCV infections were selected at random for commercial HCV genotyping (Alborg lab, Egypt), Where ten random samples were selected from the group that responded to the treatment with Sofosbuvir and ten random samples were selected from samples that did not respond to treatment with Sofosbuvir.

3 .Results

As shown in table 1a total of four hundred HCV patients were tested for HCV RNA using the reverse transcriptase polymerase chain reaction (RT-PCR) and for anti-HCV antibodies using the ELIZA Technique. Sofosbuvir was used to treat all patients.

As show in table 2 according to our findings, 354 (88.5%) of the 400 people who received sofosbuvir for Hepatitis C had treatment-related side effects, whereas just 46 (11.5%) did not .

Our findings show that 225 men (87.8%) and 129 women (89.6%) respond to sofosbuvir drug treatment, while 31 men (12.2%) and 15 women (10.4%) do not. As shown in table 3 we selected 80 patients at random, 40 who responded well to pharmacological treatment and 40 who did not. During routine clinic appointments, blood samples were collected for various analyses. Our study included two patient groups: those with anti-HCV positive and HCV RNA negative status who responded to Sofosbuvir treatment (n = 25) and those who did not respond (n = 25). (patients with positive HCV RNA and anti-HCV antibodies). Our findings revealed that there were high statistically significant differences (P. Value >= 0.005) in the level of hemoglobin (Hgb.), red blood cells (RBCs), and white blood cells (WBCs) as well as, Alanine aminotransferase enzyme (ALT), aspartate Aminotransferase (AST), Bilirubin (Bil.), C-reactive protein (CRP) and intrtleukin18 (IL-18) with patients who responded to Sofosbuvir treatment, whether before or after treatment. whether

before or after treatment. Our investigation found no significant changes (P-value < 0.005) in the level of blood platelets (PLTS) between responding patients before and after therapy. As shown in table 4 our findings revealed high statistically significant differences (P. Value >= 0.005) in the level of hemoglobin (Hgb.), red blood cells (RBCs), and white blood cells (WBCs), as well as Alanine aminotransferase enzyme (ALT) with patients who responded to Sofosbuvir treatment before after. While our study showed that there were statistically significant differences (P. Value = 0.005) in the level of blood platelets (PLTS) aspartate Aminotransferase (AST), Bilirubin (Bil.) and intrtleukin18 (IL-18) and C-reactive protein (CRP) between the responding patients, whether treatment.fter treatment. As show in table 5 our results demonstrated high significant differences (P. Value >=0.005) between non-responder and responder patients with before treatment for assessing the Alanine aminotransferase enzyme (ALT) and the level of platelets count (PLTS). The current study found significant differences (P < 0.005) between non-responder and responder patients before treatment for hemoglobin (Hgb), red blood cells (RBCs), aspartate aminotransferase (AST), and C-reactive protein (CRP), but non-significant differences (P < 0.005) for white blood cells (WBCs), bilirubin (Bil.), and intrtleukin18 (IL-18). As shown in table 6 our findings revealed high significant differences (P-value >=0.005) between non-responder and responder patients following therapy in terms of aspartate aminotransferase (AST) and C-reactive protein (CRP). While the current investigation found significant differences between non-responder and responder patients following treatment for detecting intraleukin18 (Il-18). There were no significant differences (P < 0.005) between non-responder and responder patients after therapy for measuring hemoglobin (Hgb), red blood cells (RBCs), white blood cells (WBCs), platelet count level (PLTS), Alanine aminotransferase enzyme (ALT), and bilirubin. As shown in table 7 to determine the genotypes of hepatitis C virus before treatment with Sofosbuvir, twenty samples were randomly selected from different patients, ten samples from the group that responded to treatment with Sofosbuvir, and ten samples were randomized from the samples that did not respond to treatment. with sofosbuvir. Our findings revealed that ten of the ten selected samples that responded to Sofosbuvir drug treatment were of the HCV 4 genotype, whereas eight of the ten selected samples that did not respond to Sofosbuvir drug treatment were of the HCV 4 genotype and just two were genotyped. HCV genotype 6.

Table 1: Detection of anti-HCV by ELIZA test and HCV RNA by RT-PCR for our patients

No. of patients	ELIZA results	HCV RNA
	HCV-Ab	HCV RNA
400	Positive	Positive

Table 2: Estimation of the gender-related response and non-response to sofosbuvir medication treatment.

Sex	Total NO.		Response to Sofosbuvir drug			
			Response		Non-response	
	Count	%	Count	%	Count	%
Male	256	64 %	225	87.8%	31	12.2%
Female	144	36 %	129	89.6%	15	10.4%
Total	400	100%	354	88.5%	46	11.5%

Table 3: Laboratory tests of study patients who responded to Sofosbuvir treatment both before and after treatment.

Laboratory testes in responded patients		Before Treatment with Sofosbuvir	After treatment with Sofosbuvir	T. Value	P. Value	Sig.
Hbg	Mean ±SD	11.90 ± 1.75	10.03 ± 1.03	6.196	0.000	H.S
Rbcs	Mean ±SD	4.50 ± 0.63	3.84 ± 0.93	6.414	0.000	H.S
Wbcs	Mean ±SD	1006 ± 3.02	5440 ± 2.87	11.016	0.000	H.S
Plts	Mean ±SD	291.82 ± 68.96	276.5 ± 86.37	0.889	0.379	N.S
ALT	Mean ±SD	20.25 ± 12.46	10.52 ± 2.72	5.718	0.000	H.S
AST	Mean ±SD	21.70 ± 16.78	10.78 ± 2.89	4.559	0.000	H.S
Bil.	Mean ±SD	1.63 ± 1.20	0.88 ± 0.84	5.658	0.000	H.S
Il-18	Mean ±SD	196.85 ± 54.40	124.11 ± 26.77	6.945	0.000	H.S
CRP	Mean ±SD	41.14 ± 32.28	14.43 ± 16.07	4.945	0.000	H.S
N.S: Non-Significant S: Significant H.S : Highly Significant						

Table 4: Laboratory tests of study patients who Non-responded to Sofosbuvir treatment both before and after treatment.

Laboratory testes in non-responded patients		Before Treatment with Sofosbuvir	After treatment with Sofosbuvir	T. Value	P. Value	Sig.
Hbg	Mean ±SD	11.070 ± 0.98	10.09 ± 0.98	15.272	0.000	H.S
Rbcs	Mean ±SD	4.20 ± 0.39	3.88 ± 0.37	8.206	0.000	H.S
Wbcs	Mean ±SD	9747.5 ± 1343.7	7720 ± 1416.6	8.042	0.000	H.S
Plts	Mean ±SD	345.5 ± 71.95	310.7 ± 57.08	2.038	0.048	S
Sgpt	Mean ±SD	15.82 ± 7.85	9.55 ± 1,78	5.355	0.000	H.S
Sgot	Mean ±SD	14.15 ± 17.85	9.05 ± 2.18	1.839	0.074	S
Bil.	Mean ±SD	1.36 ± 1.31	0.77 ± 0.72	3.606	0.001	S
Il-18	Mean ±SD	232.00 ± 207.22	496.52 ±561.47	-2.92	0.006	S
CRP	Mean ±SD	51.78 ± 25.57	20.24 ± 24.70	3.365	0.002	S
N.S: Non-Significant S : Significant H.S : Highly Significant						

Table 5: Comparison between responder and non-responding to Laboratory tests before treatment with Sofosbuvir.

Laboratory tests before treatment		Responded To Sofosbuvir	Non responded To Sofosbuvir	T. Value	P. Value	Sig.
Hbg	Mean ±SD	11.90 ± 1.76	11.07 ± 0.98	2.418	0.020	S
Rbcs	Mean ±SD	4.50 ± 0.63	4.20 ± 0.39	2.443	0.019	S
Wbcs	Mean ±SD	10017.5 ± 1971.2	9747.5 ± 1343.7	0.625	0.518	N.S
Plts	Mean ±SD	291.82 ± 68.96	345.52 ± 71.94	-3.290	0.002	H.S
Sgpt	Mean ±SD	20.25 ± 12.46	15.82 ± 7.85	2.027	0.005	H.S
Sgot	Mean ±SD	21.70 ± 16.78	14.15 ± 17.81	2.058	0.046	S
Bil.	Mean ±SD	1.63 ± 1.20	1.36 ± 1.31	0.905	0.31	N.S
Il-18	Mean ±SD	234.98 ± 180.93	232.00 ± 207.22	0.073	0.942	N.S
CRP	Mean ±SD	41.145 ± 32.28	51.778 ± 35.57	-2.328	0.025	S
N.S: Non-Significant S: Significant H.S: Highly Significant						

Table 6: Comparison between responder and non-responder regarding to biochemical analysis after treatment with Sofosbuvir .

Biochemical test after treatment		Responded To Sofosbuvir	Non responded To Sofosbuvir	T. Value	P. Value	Sig.
Hbg	Mean ±SD	10.03 ± 1.03	10.09 ± 0.98	-0.249	0.80	N.S
Rbcs	Mean ±SD	3.84 ± 0.39	3.88 ± 0.38	-0.416	0.679	N.S
Wbcs	Mean ±SD	7307±1812.4	7720±1416.6	-1.161	0.253	N.S
Plts	Mean ±SD	276.5 ± 86.4	310.6 ± 57.1	-1.768	0.085	N.S
Sgpt	Mean ±SD	10.52 ± 2.72	9.55 ± 1.78	1.782	0.083	N.S
Sgot	Mean ±SD	10.78 ± 2.97	9.05 ± 2.09	3.156	0.003	H.S
Bil.	Mean ±SD	0.89 ± 0.84	0.77 ± 0.72	0.576	0.568	N.S
Il-18	Mean ±SD	249.01 ± 350.42	469.5 ± 561.47	-2,170	0.035	S
CRP	Mean ±SD	14.435 ± 16.07	30.245 ± 24.70	-4.091	0.000	H.S
N.S: Non-Significant S : Significant H.S : Highly Significant						

Table 7: Detection of HCV genotypes for twenty random selected Sofosbuvir treatment.

HCV genotype by PCR	Response to Sofosbuvir	
	Response	Non-response
HCV genotype 1	0	0
HCV genotype 2	0	0
HCV genotype 3	0	0
HCV genotype 4	10	8
HCV genotype 5	0	0
HCV genotype 6	0	2

4. Discussion

Seventy percent of those infected with HCV go on to develop chronic liver disease; cirrhosis and HCC can also develop during the course of the illness. Persistent liver inflammation is strongly associated with the development of HCV-mediated liver disease, and it also increases the risk of fibrosis and an intensified immune response [16]. The most difficult public health issue Egypt has is the highest prevalence rate of hepatitis P virus (HCV) in the world. Research indicates that 9.8% of Egyptians have a current infection [18] and 14.7% of the population has HCV antibodies [17]. Prevalence rates in some age groups might reach 50%. Regarding the geographic distribution of anti-HCV in individuals between the ages of 10 and 50, the rates in the Nile Delta and Upper Egypt are 28% and 26%, respectively [19]. IL-18 increases the susceptibility of liver endothelial cells to undergo apoptosis. There have been substantial reports of evidence suggesting that IL-18 is a major factor in liver injury [20]. findings revealed that there were high statistically significant differences in the level of hemoglobin (Hgb.), red blood cells (RBCs), and white blood cells (WBCs) as well as, Alanine aminotransferase enzyme (ALT), aspartate Aminotransferase (AST), Bilirubin (Bil.), C-reactive protein (CRP) and intrtleukin18

(IL-18) with patients who responded to Sofosbuvir treatment, whether before or after treatment. whether before or after treatment. Our investigation found no significant changes in the level of blood platelets (PLTS) between responding patients before and after therapy . while Our findings revealed high statistically significant differences in the level of hemoglobin (Hgb.), red blood cells (RBCs), and white blood cells (WBCs), as well as Alanine aminotransferase enzyme (ALT) with patients who responded to Sofosbuvir treatment before after. While our study showed that there were statistically significant differences in the level of blood platelets (PLTS) aspartate Aminotransferase (AST), Bilirubin (Bil.) and intrtleukin18 (IL-18) and C-reactive protein (CRP) between the responding patients, whether before or after treatment.

In comparison to patients with chronic active hepatitis C and healthy people, the current investigation demonstrated a highly significant increase in the mean values of serum IL-18 in patients with non-complicated and complicated liver cirrhosis due to HCV. These findings were in agreement with Jia et al. **[21]** who reported that patients with chronic hepatitis C had significantly higher serum levels of IL-18 compared without symptoms HCV carriers**[21]**.

Our findings revealed high significant differences between non-responder and responder patients following therapy in terms of aspartate aminotransferase (AST) and C-reactive protein (CRP). While the current investigation found significant differences between non-responder and responder patients following treatment for detecting intraleukin18 (Il-18). There were no significant differences between non-responder and responder patients after therapy for measuring hemoglobin (Hgb), red blood cells (RBCs), white blood cells (WBCs), platelet count level (PLTS), Alanine aminotransferase enzyme (ALT), and bilirubin. These findings were in agreement with Nadia *et al*., (2013) who found The difference in ALT and AST levels between responders and non-responders was not statistically significant. [23]. Persistently normal ALT is typically described as ALT levels in the normal range during a 6-to-12-month period [24]

References

1. Pachiadakis J, Pollara G, Chain BM and Naoumov NV (2005): Is hepatitis C virus infection of dendritic cells, a mechanism facilitating viral persistence? Lancet Infect. Dis; 5: 296-304

2. Thakeb AF, Omar MM, Ishak YS and El-Awady M (2003): Randomized controlled trial of peg elated interferon alpha-24 plus ribavirin for hepatitis C virus genotype 4 among Egyptian patients. Hepatol.; 38 (4): 278A.

3. Gerlach T, Gruener N, Jung MC, Shrout W, Schirren A, Backmund M and diepolder H (2003): Characterization of novel CD4+, T-cell epitopes in acute self-limited HCV infection. Hepatol.; 38 (4): 172-178.

4. Delpuech O, Buffelo-Legullou D, Feray C and Petit MH (2000): 10th international symposium on viral hepatitis and liver diseases ;(5), 114 C.

5. Esmat G (2013): Hepatitis C in the Eastern Mediterranean Region. Eastern Mediterranean Health Journal 19: 587-588.

6. El-Zanaty F, Way A (2009): Egypt Demographic and Health Survey 2008. Ministry of Health, El-Zanaty and Associates, and Macro International, Cairo, Egypt.

7. Biet F, Locht C and Kremer L (2002): Immunoregulatory functions of interleukin 18 and its role in defense against bacterial pathogens. J. Mol. Med.; 80: 147- 162.

8. Lawitz E, Mangia A, Wyles D, Rodriguez-Torres M, Hassanein T, et al. (2013): Sofosbuvir for previously untreated chronic hepatitis C infection. New England Journal of Medicine 368: 1878-1887.

9. Abdel-Wahab MF, Zakaria S, Kamel M, Abdel-Khaliq MK, Mabrouk MA, et al. (1994): High seroprevalence of hepatitis C infection among risk groups in Egypt. The American journal of tropical medicine and hygiene 51: 563-567.

10. Hepatitis C (2016): GAR-WHO, Geneva, Switzerland, USA.

11. Kaser, D., Novick, M., Rubeinstein, B., Siegmund, B., Enrich, R. and Koch, W. (2002): Interferon-α induces interleukin-18 binding protein in chronic hepatitis C patients. Clin Exp Immunol., 129(2):332–338.

12. Zahida, B. and Charles, S. B. (2007): Adult systemic cat scratch disease associated with therapy for hepatitis C. BMC Infect Dis. 7: 8.

13. Pai, C.K., Shu-Chu, S.H. and Ta-Jen, W. (2006): Review: Serum C - reactive protein as a Marker for Wellness Assessment. Annals of Clinical & Laboratory Science, 36(2): 163- 169.

14. Riquelme, A., Arrese, M., Soza, A., Morales, A., Baudrand, R., Perez-Ayuso, R.M., Gonzalez, R., Alvarez, M., Hernandez, V., Garcia-Zattera, M.J., Otarola, F., Medina, B., Rigotti, A., Miquel, J.F., Marshall, G. and Nervi, F. (2009): Non-alcoholic fatty liver disease and its association with obesity, insulin resistance and increased serum levels of C-reactive protein in Hispanics. Liver Int; 29:82–88.

15. .Kogiso, T., Moriyoshi, Y., Shimizu, S., Nagahara, H. and Shiratori, K. (2009): High-sensitivity C-reactive protein as a serum predictor of nonalcoholic fatty liver disease based on the Akaike Information Criterion scoring system in the general Japanese population. J Gastroenterol; 44:313–321.

16. Gravitz L.(2011): Introduction: a smouldering public-health crisis. *Nature* 2011; 474: S2–S4.

17. Esmat G (2013): Hepatitis C in the Eastern Mediterranean Region. Eastern Mediterranean Health Journal 19: 587-588.

18. El-Zanaty F, Way A (2009): Egypt Demographic and Health Survey 2008. Ministry of Health, El-Zanaty and Associates, and Macro International, Cairo, Egypt.

19. Wahid D, Mostafa KM, Gamal E, Manal ES, Arnaud F, et al. (2008): Egyptian National Control Strategy for Viral Hepatitis. National Committee for the Control of Viral Hepatitis, Egypt.

20. Marino E, Cardier JE. (2003): Differential effect of IL-18 on endothelial cell apoptosis mediated by TNF-alpha and Fas (CD95). *Cytokine*; 22:142–148.

21. Jia H, Du J, Zhu S, Ma Y, Cai H (2003): Clinical observation of serum IL-18, IL-10 and sIL-2R levels in patients with chronic hepatitis C pre-and post antiviral treatment. *Clin Med J* ; 116: 605-608.

22. Sharma A, Chakraborti A, Das A, Dhiman RK, Chawla Y. (2008): Elevation of interleukin-18 in chronic hepatitis C: implications for hepatitis C virus pathogenesis. *Immunology* ; 128: 514–522.

23. Mohran, Z.Y., Ali-Eldin, F.A. and Abdel Aal, H.A. (2011) Serum

interleukin-18: does it have a role in the diagnosis of hepatitis C virus related hepatocellular carcinoma? Arab J Gastroenterol.;12(1):29-33.

24. Strader D.B., Wright T., Thomas D.L., Seef L.B. and AASLD (2004): Diagnosis, management and treatment of hepatitis C. Hepatol; 39:1147-71.