ORIGINAL ARTICLE

Association of Serum Interlukin-6 and Transforming Growth Factor Beta with Response to Antiviral Therapy for Chronic Hepatitis C Patients

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ABSTRACT

Background: *Hepatitis C virus (HCV) infection is an important contributor for acute and chronic liver disorders. Drug acting antivirals (DAAs) are compounds; as sofosbuvir and daclatasvir; target HCV replication cycle to inhibit viral replication and promote gradual clearance of the virus by death of infected cells. Pro-inflammatory cytokines; as interleukin-6 (IL-6) and Transforming growth factor β (TGF- β); are induced by HCV viral dsRNA and proteins.* ***Objective:*** *To declare if there is a relationship between serum IL-6 and TGF- β levels and response to DDAs in chronic HCV patients.* ***Methodology:*** *Forty patients with Chronic HCV were involved. PCR for detection of viral activity was performed before treatment, 12 weeks after the end of treatment. According to PCR results, patients were divided into: 2 non-responders and 33 sustained virologic responders (SVR). 14 healthy individuals were included as a control group. Total bilirubin, direct bilirubin, albumin, AST, ALT, ALP, urea, creatinine, prothrombin time, prothrombin concentration and international normalization ratio (INR) were reported. The serum levels of IL-6 and TGF-β were measured using ELISA kits.* ***Results:*** *In patients with SVR and control groups, mean levels of ALT, AST, albumin, total bilirubin and ALP were within normal range. In naïve CHC patients, the mean levels of ALT, AST and total bilirubin were increased, but mean levels of albumin and ALP were within normal range. IL-6 and TGF-β levels decreased significantly after treatment in SVR group.* ***Conclusion:*** *Virological response during HCV therapy was associated with decrease in IL-6 and TGF-β levels.*

INTRODUCTION

Hepatitis C virus (HCV) infection is an important contributor for both acute and chronic liver disorders [1]. According to the World Health Organization (WHO), about 71 million patients are infected with HCV and may expose to develop catastrophic liver disorders as liver cirrhosis and hepatocellular carcinoma (HCC); both of which may become fatal [2].

Hepatitis C virus (HCV) infection is prevalent at the highest rate in Egypt globally, where it is linked to serious illness and financial costs [3]. So, it became a national medical concern to eradicate HCV [4]. Hepatitis viruses cause 1.34 million deaths, which is similar to other diseases as TB and AIDS[1].

HCV prevalence is 14.7% in Egypt; nearly 1 among 10 people; their age range between 15 at 59 have HCV which is extremely contagious. The prevalence of HCV ranges between 1% and 2% in other nations and may reach in some areas to 3% [5].

According to the Ministry of Health, hepatitis C prevalence rate fell from 7 % in 2018 to 2% in 2021 [6]. According to Khaled Mogahed, the Ministry of Health's spokesperson, on February 6, 2021, 70 million adults over the age of 18 underwent examinations as part of the Egyptian President Abdel Fatah al-Sisi motivate to eliminate Hepatitis C and diagnose the diseases early [6].

There are eight genotypes of HCV that vary in accordance to geographic areas, particular symptoms, and response to treatment [7,8]. Nearly 90% of HCV isolates from Egypt are subtype 4a, which is less responsive to interferon therapy compared to other genotypes [9].

Drug acting antivirals (DAAs) are compounds that specifically target nonstructural viral proteins, preventing viral infection and replication. The DAAs agents now target HCV replication cycle to inhibit viral replication and promote gradual clearance of the virus by death of infected cells [10].

In some populations, sustained virological response (SVR) rates for HCV treatment with (DAA) regimens have reached 90-95% [11]. This is explained by the fact that immune responses of the host are released from

suppressor effect of the virus by DAAs, enhancing the effectiveness of HCV treatments [12].

Surprisingly, numerous studies have demonstrated that DAA can not only safely and effectively eradicate HCV but also achieve some additional advantages when compared to interferon, like liver function damage repairing, recovering from metabolic impairment, and restoring immunity dysfunction due to HCV infection. Additionally, the number of patients who experience adverse effects is significantly lower [13].

When compared to people without the disease, studies show that people with chronic hepatitis C have higher serum levels of cytokine [14].

Pro-inflammatory chemokines and cytokines are induced by HCV viral dsRNA and proteins. The core protein of HCV activates the STAT3 (signal transducer and activator of transcription proteins) signaling pathway inducing production of inflammatory cytokines[15].

Interleukin 6 (IL-6) has anti-inflammatory and pro-inflammatory properties. Human IL-6 is a glycosylated protein, produced by T cells, B cells, fibroblasts, and macrophages, weighting 26 kD molecularly and located at 7p21 chromosome. IL-6's progenitor peptide has 212 amino acids. Important transcriptional organizing elements in the IL-6 gene promoter region are regulated by nuclear factor kappa B (NF-κB), activating protein-1 (AP1), and other proteins [16,17].

The primary role of IL-6 is hepatic reaction to infections and generalized inflammation [18].

By affecting the polarization and functioning of Th-1 cells and the lytic capacity of CD8 T cells, over-expression of IL-6 may, through a variety of mechanisms, promote viral persistence and chronicity[19].

Transforming growth factor beta (TGF- β) is a cytokine with profibrogenic, immunosuppressive and anti-inflammatory properties and its over-expression is tangled in a variety of processes of liver disease. To maintain homeostasis of tissues, all of these actions must be balanced [20].

The TGF- β is produced by regulatory T lymphocytes (T-regs), which also activate macrophages and regulate the activity of many immune cells using various mechanisms. TGF- β prevents T lymphocyte proliferation and activation, so it prevents CD4+ T lymphocytes from differentiating into Th1 cells and CD8+ T lymphocytes from becoming cytotoxic [21].

The aim of this work was to declare if there is a possible relationship between serum cytokine levels and response to DDAs in chronic hepatitis C patients through assessment of serum levels of IL-6 and TGF- β before (naive) and after successful treatment (sustained responders) with (sofosbuvir +daclatasvir) for 3 months regimen in patients with chronic hepatitis C and also through evaluation of the relationship between levels of IL-6 and TGF-β and response to DAAs.

METHODOLOGY

Study design and population

This study was performed at the Department of Medical Microbiology and Immunology, Faculty of Medicine and at the Outpatient Clinic of Tropical Medicine Department at AL-Rajhi-Liver Hospital, Assiut University. This cohort study (Gov ID: NCT03882307) was conducted on a total of 40 chronic HCV patients, presenting to the Outpatient Clinic of Tropical Medicine Department at AL-Rajhi-Liver Hospital, Assiut University from September 2020 to June 2021, to be treated with sofosbuvir (SOF) (400 mg once per day) and daclatasvir (DCV) (60 mg once per day) for 3 months. Control group included 14 healthy subjects. PCR for detection of viral activity was performed before treatment, 12 weeks after the end of treatment.

Forty patients fulfilled the inclusion and exclusion criteria.

According to The Egyptian Treatment Guidelines For HCV; inclusion criteria were HCV RNA positivity, treatment-naïve, age from 18 to 70 years, any body mass Index (BMI), and all fibrosis stages. Exclusion criteria were serum albumin less than 2.8 g/dl, direct serum bilirubin greater than 2 mg/dl, serum creatinine > 2.5 mg/dL, platelets count less than 50 000/mm^3, international normalization ratio (INR) greater than or equal to 1.7, ascites or history of ascites, hepatocellular carcinoma (HCC), hepatic encephalopathy or history of hepatic encephalopathy, and inability to use effective contraception or pregnancy[22].

All included patients were positive for HCV antibodies by ELISA and HCV RNA by real time PCR (RT-PCR).

Control group included 14 healthy controls ;6 female and 8 male patients. Healthy blood donors were included as controls; they were attending blood bank of Assiut University Hospital during the study period. They were negative for known serologic markers of hepatitis (B & C) including hepatitis B surface antigen and antibodies to HCV.

Ethical aspect

After receiving informed consent, patients were authorized to participate in the study. The standard of patients' care got in the hospital was never impacted by their unwillingness to participate in the study. The study protocol was approved by the local ethical committee in 7/4/2019 with (IRB) no:17100703.

All patients were subjected to history taking, clinical examination, abdominal ultrasonography, Liver function tests and measurement of IL-6 and TGF-β

The serum levels of IL-6 and TGF-β were measured from all research groups using Enzyme Linked Immunosorbent Assay kits (Bioassay Technology

Laboratory, Shanghai Korain Biotech Co., Ltd.) according to manufacturer's instructions.

Statistical analysis:

For each group; the mean and standard deviation values were computed. Using the Shapiro-Wilk and Kolmogorov-Smirnov tests to determine normality, a non-parametric (not normal) distribution was found. Continuous data were expressed as the mean ± SD and range, and categorical data were expressed as number and percentage. Kruskal Wallis test was used to compare between more than two groups in non-related samples. Mann Whitney test was used to compare between two groups in non-related samples. Spear man test was used for correlation coefficients. The significance level was set at $P < 0.05$. Statistical analysis was performed with IBM® SPSS® Statistics Version 26 for windows.

RESULTS

Patients were divided into3 groups:

1. Group I included all 40 chronic HCV-infected patients before initiation of DAA treatment (Naïve CHC); 22 female and 18 male patients.
2. Group II included 33 patients with sustained viral response (SVR) 3 months from the end of treatment; 19 female and 14 male patients.
3. Group III included 2 patients with positive HCV RNA 3 months from the end of treatment (non-responder); 2 male patients.

Demographic data of patients and control groups

Ages and sex of patients infected with HCV were reported during collection of samples. There were 2 male non-responders, the mean age ±SD (58.5± 7.77). The mean age of SVR patients was younger than non-responders& the response to treatment was more in females (57.58%) than in males with no statistically significant difference as shown in table (1).

Table 1: Age & Sex characteristics of naïve, SVR and control groups

	Naïve(No.=40)		**SVR(No.=33)**		**Control (No.=14)**		**p-value**
	Mean±SD		Mean±SD		Mean±SD		
Age	40.05±14.6		39.96±14.98		32.5±9.08		0.317
Sex	No.	%	No.	%	No.	%	
Male	18	45.00	14	42.42	8	57.15	0.642
Female	22	55.00	19	57.58	6	42.85	

Kruskal Wallis test was used to compare between all groups about age variable, where used **Chi-square** test to compare between all groups.

In patients with SVR and control groups, mean levels of ALT, AST, albumin, total bilirubin and ALP were within normal range. In naïve CHC patients, the mean levels of ALT, AST and total bilirubin were increased, but mean levels of albumin and ALP were within normal range.

There was a highly statistically significant difference between naïve and SVR and between naïve and control for total bilirubin, direct bilirubin, AST, ALT and creatinine

There was a statistically significant difference between naïve and SVR for ALP and prothrombin time, between naïve and control for urea and prothrombin time and between SVR and control for AST and creatinine as shown in table (2).

Table 2: Laboratory data of naïve, SVR and control groups

Laboratory variables	**Normal value**	**Naïve CHC (n=40)**	**SVR (n=33)**	**Control (n=14)**	**P1- value**	**P2-value**	**P3-value**
		Mean±SD	**Mean±SD**	**Mean±SD**			
PCR (IU/ml)	Nil	551675±166362.7	0±0	0±0	-	-	-
Total Bilirubin (mg/dl)	0.1-1.2	1.78±0.996	0.73±0.29	0.597±0.29	**<0.001****	**<0.001****	0.139
Direct Bilirubin (mg/dl)	0.1-0.3	0.93±0.62	0.23±0.12	0.18±0.059	**<0.001****	**<0.001****	0.368
Albumin (g/dl)	3.5-5.5	4.68±0.48	4.55±0.495	4.37±0.62	0.227	0.127	0.401
AST (U/L)	10-40	50.03±21.23	18.24±4.67	13.18±2.55	**<0.001****	**<0.001****	**0.003***
ALT (U/L)	7-55	79.97±15.74	13.1±4.32	11.64±2.21	**<0.001****	**<0.001****	0.196
ALP (U/L)	44-147	72.55±13.11	64.88±10.18	67.07±16.59	**0.007***	0.155	0.981
Urea (mg/dl)	8-20	15.295±2.69	14.21±2.7	12.8±1.69	0.089	**0.004***	0.133
Creatinine (mg/dl)	0.7-1.3	1.13±1.18	0.85±0.079	0.78±0.05	**<0.001****	**<0.001****	**0.003***
Prothrombin time (seconds)	9.5-13.5	12.22±1.002	11.46±0.99	11.69±0.84	**0.002***	**0.029***	0.442
Prothrombin Concentration	75-140	98.76±13.72	97.16±12.88	95.54±10.61	0.657	0.622	0.935
INR	0.9-1.3	0.94±0.08	0.96±0.07	0.94±0.065	0.185	0.945	0.277

P1, P2 & P3 calculated by **Mann Whitney** test
P1-value between naïve CHC and SVR
P2-value between naïve CHC and control
P3-value between SVR and control

In non - responders, the mean levels of ALT were increased, while the mean levels of AST, albumin, ALP, total bilirubin and other laboratory variables were within normal range as shown in table (3).

Table 3: Laboratory data of non-responders.

Laboratory variables	Normal value	Mean±SD
PCR (IU/ml)	Nil	680000± 49497.48
Total Bilirubin (mg/dl)	0.1-1.2	1.1 ±0.28
Direct Bilirubin (mg/dl)	0.1-0.3	0.21±0.02
Albumin (g/dl)	3.5-5.5	4.8±0.14
AST (U/L)	10-40	39.45±1.2
ALT (U/L)	7-55	82.75±8.27
ALP (U/L)	44-147	83.05±7.28
Urea (mg/dl)	8-20	13.35±0.78
Creatinine (mg/dl)	0.7-1.3	0.83±0.085
Prothrombin time (seconds)	9.5-13.5	11.2±0.99
Prothrombin Concentration	75-140	106.3±9.19
INR	0.9-1.3	1.2±0.028

Assessment of serum IL-6 and TGF-β levels using ELISA

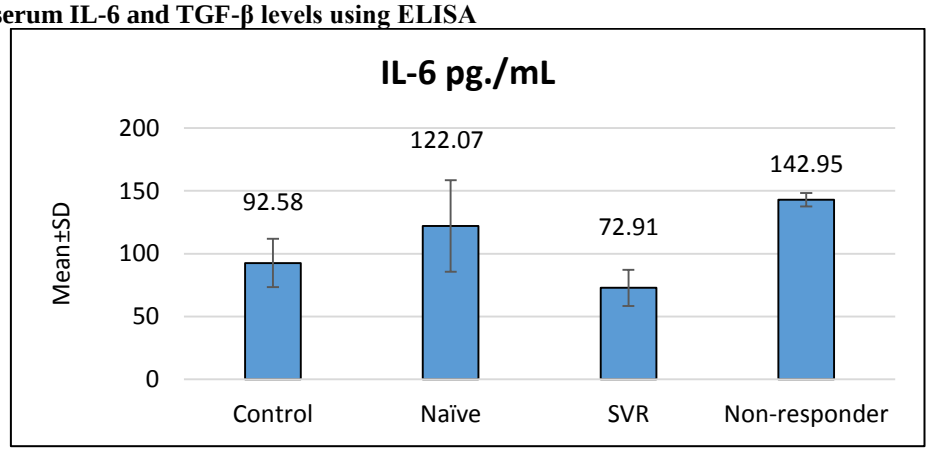

Fig. 1: Mean concentration of serum IL-6 in studied groups.

The mean concentration of serum IL-6 was the highest in non-responder group then Naïve CHC group then control with the least mean value detected in SVR as shown in fig. (1).

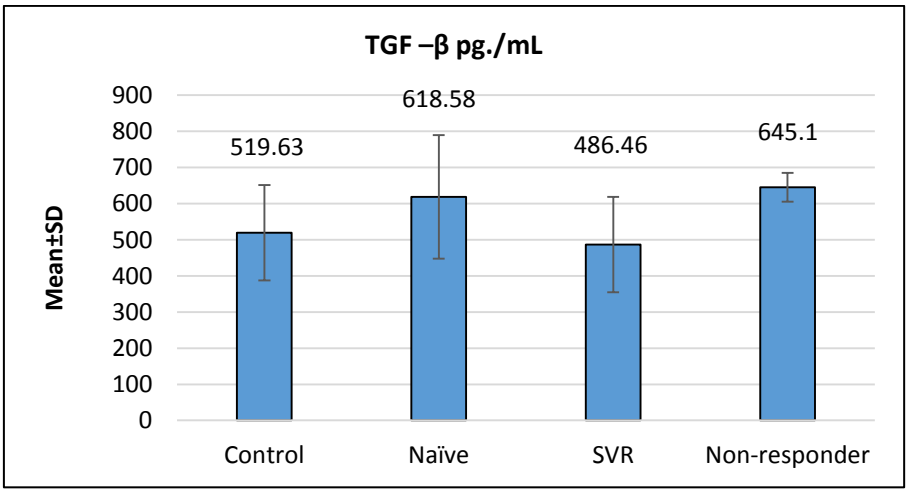

Fig. 2: Mean concentration of serum TGF-β in studied groups.

The mean concentration of serum TGF –β was the highest in non-responder group then Naïve CHC then control group with the least mean value detected in SVR as shown in fig. (2).

There was a highly statistically significant difference for IL-6 and TGF-β between naïve, SVR and control groups as shown in table (4).

Table 4: Serum IL-6 and TGF –β levels in naïve, SVR and control groups

	Naïve No=40	**SVR No=33**	**Control No=14**	**p-value**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	
IL-6 (pg./mL)	122.07±36.32	72.91±14.396	92.58±19.19	**<0.001****
TGF –β (pg./mL)	618.58±171.039	486.46±131.85	519.63±131.97	**0.001****

Kruskal Wallis test was used to compare between all groups.
** There is a high significant statistical difference (p<0.001)

Serum IL-6 and TGF –β levels of 2 non-responders were 142.95±5.44 and 645.1± 39.88 respectively.

There was a high statistically significant difference for IL-6 between control and SVR, between SVR and naïve (p<0.001) and a statistically significant difference between control and naïve (p<0.05). There was a high statistically significant difference for TGF-β between SVR and naïve (p<0.001), as shown in table (5).

Table 5: Comparison between control, SVR and naïve groups as regard serum IL-6 and TGF-β levels

Il-6 (pg./ml)	**Control (92.58±19.19)**	**SVR (72.91±14.396)**	**Naïve (122.07±36.32)**
Control	-		
SVR	**0.001****	-	
Naïve	**0.009***	**<0.001****	-
TGF-β (pg./ml)	**Control** (519.63±131.97)	**SVR** (486.46±131.85)	**Naïve** (618.58±171.039)
Control	-		
SVR	0.213	-	
Naïve	0.058	**<0.001****	-

Mann-Whitney test was used for comparison between each two groups
* There is a significant statistical difference (p<0.05)
** There is a high significant statistical difference (p<0.001)

There was a positive significant correlation between prothrombin concentration and IL-6 and between AST and IL-6 among control group as shown in table (6).

Table 6: Correlation of serum IL-6 level with laboratory data of control, naïve and SVR

Laboratory variables	**IL-6**					
	Control(n=14)		**Naïve CHC (n=40)**		**SVR (n=33)**	
	r	**P**	**r**	**P**	**R**	**P**
PCR (IU/ml)	-	-	-0.306	0.054	-	-
Total Bilirubin (mg/dl)	-0.031	0.916	0.092	0.572	-0.046	0.799
Direct Bilirubin (mg/dl)	0.410	0.145	0.082	0.615	0.083	0.644
Albumin (g/dl)	-0.130	0.658	0.086	0.600	-0.028	0.878
AST (U/L)	0.579	0.030*	0.035	0.829	-0.011	0.952
ALT (U/L)	0.373	0.189	-0.202	0.211	0.010	0.955
ALP (U/L)	-0.178	0.542	-0.174	0.283	0.154	0.392
Urea (mg/dl)	-0.486	0.078	0.053	0.747	-0.276	0.120
Creatinine (mg/dl)	0.272	0.346	-0.213	0.186	-0.069	0.704
Prothrombin time (seconds)	-0.029	0.923	-0.049	0.764	0.292	0.099
Prothrombin Concentration	0.657	0.011*	-0.137	0.399	-0.289	0.102
INR	0.148	0.613	-0.026	0.872	-0.314	0.075

Used **Spear man** correlation coefficients.
* There is a significant statistical difference (p<0.05)
r = correlation

There was a negative significant correlation between AST and TGF-β and between INR and TGF-β among control group and there was a negative significant correlation between AST and TGF-β among naïve group as shown in table (7).

Table 7: Correlation of serum TGF-β level with laboratory data of control, naïve and SVR

Laboratory variables	TGF-β					
	Control (n=14)		Naïve CHC (n=40)		SVR (n=33)	
	r	P	r	P	r	P
PCR (IU/ml)	-	-	0.149	0.359	-	-
Total Bilirubin (mg/dl)	-0.131	0.656	-0.277	0.083	-0.182	0.311
Direct Bilirubin (mg/dl)	-0.122	0.678	-0.119	0.466	-0.122	0.499
Albumin (g/dl)	-0.165	0.572	-0.023	0.889	0.058	0.750
AST (U/L)	-0.541	0.046*	-0.331	0.037*	0.312	0.077
ALT (U/L)	0.022	0.940	-0.148	0.362	-0.106	0.557
ALP (U/L)	-0.427	0.128	-0.180	0.267	-0.029	0.872
Urea (mg/dl)	0.345	0.226	-0.107	0.512	-0.038	0.833
Creatinine (mg/dl)	-0.086	0.769	-0.184	0.255	-0.091	0.615
Prothrombin time (seconds)	-0.447	0.109	0.066	0.686	-0.165	0.359
Prothrombin Concentration	-0.358	0.208	0.084	0.607	0.104	0.565
INR	-0.577	0.031*	-0.139	0.392	0.088	0.627

Used **Spear man** correlation coefficients.
* There is a significant statistical difference ($p<0.05$)
r = correlation

DISCUSSION

In Egypt, HCV infection is regarded as a serious medical problem. For the treatment of HCV, a dual regimen of sofosbuvir and ribavirin is currently accessible[23] .

The first aim of this study was to measure serum levels of IL-6 and TGF-β in naïve CHC (group I), patients who received direct antiviral therapy showing SVR (group II), non-responders (group III) and healthy controls (group IV), aiming to prove whether there is association between these cytokines and the response to DAA in HCV infection.

The present findings showed that the mean age of the HCV naïve patients was higher than that of patients with SVR and controls but the mean age of the HCV non-responder patients was the highest.This may be because the disease takes a long time to develop and become complicated [24].

According to the gender of HCV infected individuals, this virus affects females more than males (female 55% vs male 45%). The current study agreed with Jamil & Ahmad [25] and Muslim [26] from Baghdad and Wasit governorates respectively , while higher distribution of HCV infection in male than in females was reported in other studies [27].

In the present study, laboratory data were collected. ALT levels were higher in HCV naïve patients than patients with SVR and controls but ALT levels were the highest in HCV non-responder patients. AST levels were higher in HCV naïve patients than patients with SVR and controls , while albumin was in normal ranges in all studied groups.These findings were consistent with those reported by Hetta et al. [28] who stated that ALT and AST levels were higher in naïve CHC followed by SVR group and lastly controls . This could be explained by the fact that albumin reflects nutritional condition synthetic function of the liver whereas AST and ALT levels during HCV infection indicate active hepatic inflammation and tissue destruction [29].

In the current study , serum IL-6 level was measured by indirect ELISA and the level was the highest in non-responders, then naïve HCV group , then controls with the least mean value detected in SVR with statistically significant differences between these values. Baseline IL-6 levels were significantly higher in patients than control group. This finding was in harmony with the other studies [30]. Also, studies by Mohamed et al. [31] showed that following ribavirin and pegylated IFN- α therapy, non-responders had significantly higher serum IL6 levels than responders.This may be explained by altering theraputic response by IL-6 levels through activating STAT3 via phosphorylation in hepatic stellate cells and by boosting their viability and proliferation. The proapoptotic and antiviral genes are induced after IFN- α activates STAT3, which may explain IFN- α 's anticancer and antiviral effects in the liver [32].

In the current study, serum TGF-β level was measured by indirect ELISA and the level was the

highest in non-responders, then naïve CHC group , then controls with the least mean value detected in SVR .Also, serum TGF-β level was only significantly higher in naïve CHC patients vs. SVR (P value < 0.001).

The HCV patients had higher mean TGF- β concentrations than the control group. This indicated that hepatocytes damage or inflammation during HCV infection is caused by immune response suppression, which is symbolised by T-cell escape. Therefore, this inhibitory activity may worsen the condition and cause chronicity that leads to HCC [33].

Pereira et al. [22] observed that T-regs have been revealed to block T-cell immunological responses by inducing cytokines secretion, particularly TGF- β which act as an effector cytokine explainning why HCV persists over time.

Another finding made by Mehmedović et al. [34] found that this cytokine was regarded as a crucial indicator for evaluating fibrosis and inflammatory activity in the assessment of chronic hepatic damage. As well as, Presser et al. [35] demonstrated that replication of HCV-RNA is positively regulated by TGF-β. Also, studies of Benzoubir et al. [36] and Schon & Weiskirchen [33] concluded that HCV core protein increases the level of active TGF-β in hepatoma cells of mutant mice.

In our study, multi-comparison was done for IL-6 and TGF-β in control, naïve and SVR groups, correlation between IL6 and TGF- β serum level with laboratory data was also done.There was a significant positive correlation between IL-6 level and each of AST and prothrombin concentration in control group.This is in harmony with Nakagawa et al. [37] while in disagreement with Mohamed et al. [31] who reported a a negative correlation between the serum IL6 level and AST and Elgonimy et al. [38] who reported no correlation between IL6 level and prothrombin concentration. In the present study, there was a significant negative correlation between TGF-β level and each of AST and INR in control group and between TGF-β level and AST in naïve CHC group .This is in disagreement with Abdeen et al. [39] and Elbanan et al. [40] who reported a high significant correlation between TGF-β level and each of ASTand INR respectively. The disparity in sample sizes could be the cause of this disagreement in correlation. [39]

CONCLUSION

Virological response during HCV therapy was associated with decrease in IL-6 level. The mean levels of IL-6 and TGF-β were significantly higher in patients than SVR and control. The results have significant implications on the role of DAA on the levels of IL-6 and TGF-β. IL-6 and TGF-β could be used for prediction of HCV response to SOF/DCV therapy, through the significant statistical correlation between SVR and naïve (p value **<0.001**)**. The reduction in the serum IL-6 levels and TGF-β following successful anti-viral treatment were associated with the clearance of infection.

Conflict of Interest: The authors report no conflicts of interest in this work.

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REFERENCES

1. Bartenschlager R, Baumert TF, Bukh J, et al .Critical challenges and emerging opportunities in hepatitis C virus research in an era of potent antiviral therapy: Considerations for scientists and funding agencies. *Virus Res* 2018;248:53-62.
2. WHO . (2017). Global hepatitis 2017 report.
3. Polaris Observatory HCV Collaborators. Global prevalence and genotype distribution of hepatitis C virus infection in 2015: a modelling study. *Lancet Gastroenterol Hepatol* 2017;2(3):161-76.
4. El-Zanaty F & Way A. Knowledge and prevalence of hepatitis C. *EDHS* 2019 ;9(6):94-103.
5. Hassanin A, Kamel S, Waked I, Fort M. Egypt's Ambitious Strategy to Eliminate Hepatitis C Virus: A Case Study. *Glob Health Sci Pract* 2021; 9(1):187-200.
6. Egypt; The ministry of health (MOH). 2021; Egypt Today.
7. Borgia SM, Hedskog C, Parhy B, et al. Identification of a Novel Hepatitis C Virus Genotype From Punjab, India: Expanding Classification of Hepatitis C Virus Into 8 Genotypes. *J Infect Dis* 2018;218(11):1722-9.
8. Spearman CW, Dusheiko GM, Hellard M, Sonderup M. Hepatitis C. *Lancet* 2019; 394(10207):1451-66.
9. Rahman El-Zayadi A, Abaza H, Shawky S, Mohamed MK, Selim OE, Badran HM. Prevalence and epidemiological features of hepatocellular carcinoma in Egypt-a single center experience. *Hepatol Res* 2001;19(2):170-9.
10. Pawlotsky JM. New hepatitis C virus (HCV) drugs and the hope for a cure: concepts in anti-HCV drug development. *Semin Liver Dis* 2014;34(1):22-9.
11. Sulkowski MS, Gardiner DF, Rodriguez-Torres M, et al. Daclatasvir plus sofosbuvir for previously treated or untreated chronic HCV infection . *N Engl J Med* 2014;370(3):211-21.
12. Lin JC, Habersetzer F, Rodriguez-Torres M, et al. Interferon γ-induced protein 10 kinetics in treatment-naive versus treatment-experienced patients receiving interferon-free therapy for

hepatitis C virus infection: implications for the innate immune response. *J Infect Dis* 2014;210(12):1881-5.

13. Amaddeo G, Nguyen CT, Maillé P, et al. Intrahepatic immune changes after hepatitis c virus eradication by direct-acting antiviral therapy. *Liver Int* 2020;40(1):74-82.
14. Baskic D, Vukovic VR, Popovic S, et al. Cytokine profile in chronic hepatitis C: An observation. *Cytokine* 2017;96:185-8.
15. Basu A, Meyer K, Lai KK, et al. Microarray analyses and molecular profiling of Stat3 signaling pathway induced by hepatitis C virus core protein in human hepatocytes. *Virology* 2006;349(2):347-58.
16. Lan T, Chang L, Wu L, Yuan YF. IL-6 Plays a Crucial Role in HBV Infection. *J Clin Transl Hepatol* 2015;3(4):271-6.
17. Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. *Cold Spring Harb Perspect Biol* 2014;6(10):a016295.
18. Yao X, Huang J, Zhong H, et al. Targeting interleukin-6 in inflammatory autoimmune diseases and cancers. *Pharmacol Ther* 2014;141(2):125-39.
19. Velazquez-Salinas L, Verdugo-Rodriguez A, Rodriguez LL, Borca MV.The Role of Interleukin 6 During Viral Infections. *Front Microbiol* 2019; 10:1057.
20. Bissell DM, Wang SS, Jarnagin WR, Roll FJ. Cell-specific expression of transforming growth factor-beta in rat liver. Evidence for autocrine regulation of hepatocyte proliferation. *J Clin Invest* 1995;96(1):447-55.
21. Kelly A, Houston SA, Sherwood E, Casulli J, Travis MA. Regulation of Innate and Adaptive Immunity by TGFβ. *Adv Immunol* 2017;134:137-233.
22. National Committee for Control of Viral Hepatitis. (http://www.nccvh.org.eg/).
23. Aboud A, Abdel-Motaleb M, Fahmy E. Evaluation of Sofosbuvir plus Ribavirinin Treatment of Cirrhotic Hepatitis C Patients: An Egyptian Study. *EJMM* 2019; 28(1): 15-22.
24. Niu Z, Zhang P, Tong Y. Age and gender distribution of Hepatitis C virus prevalence and genotypes of individuals of physical examination in WuHan, Central China. *Springerplus*. 2016;5(1):1557.
25. Jamil NF & Ahmad MJ. Seroprevalence of Hepatitis C and Associated Risk Factors in Hemodialysis Units in Baghdad. *Iraqi J Com Med* 2015;28(4):162-7.
26. Muslim TM. Epidimiologic Study of Hepatitis B and C Virus Among Thalassemia Patients in Wasit Governarate/Iraq. *Al-Taqani* 2014;27(2): E1-E6.
27. Fedeli U, Avossa F, Ferroni E, De Paoli A, Donato F, Corti MC. Prevalence of chronic liver disease among young/middle-aged adults in Northern Italy: role of hepatitis B and hepatitis C virus infection by age, sex, ethnicity. *Heliyon* 2019;5(7):e02114.
28. Hetta HF, Mekky MA, Khalil NK, et al. Association of colonic regulatory T cells with hepatitis C virus pathogenesis and liver pathology. *J Gastroenterol Hepatol* 2015;30(10) :1543-51.
29. Kostadinova L, Shive CL, Zebrowski E, et al. Soluble Markers of Immune Activation Differentially Normalize and Selectively Associate with Improvement in AST, ALT, Albumin, and Transient Elastography During IFN-Free HCV Therapy. *Pathog Immun* 2018;3(1):149-63.
30. Mohamed AA, El-Toukhy N & Reyad EM. Serum Interleukin-6 Concentration Associated with Response to Therapy for Chronic Hepatitis C Patients. *J Gastroenterol Hepatol Res* 2017;6(4): 2405-10.
31. Mohamed AA, Afifi EA & El-Awady RR.Correlation between Serum Levels of TNFR and IL6 with Treatment Response to Pegylated Interferon and Ribavirin Therapy in Chronic Hepatitis C Egyptian Patients. *J O J Immuno Virology* 2015; 1(2):e555559
32. Gao B. Cytokines, STATs and liver disease. *Cell Mol Immunol* 2005;2(2):92-100.
33. Schon HT, Weiskirchen R. Immunomodulatory effects of transforming growth factor-β in the liver. *Hepatobiliary Surg Nutr* 2014;3(6):386-406.
34. Mehmedović A, Mesihović R, Prnjavorac B, et al. Non-invasive liver fibrosis markers: use of serum levels of cytokines IL 1α and TGF ß1 in management of chronic liver diseases. *Med Glas (Zenica)* 2013;10(1):20-7.
35. Presser LD, Haskett A, Waris G. Hepatitis C virus-induced furin and thrombospondin-1 activate TGF-β1: role of TGF-β1 in HCV replication. *Virology* 2011;412(2):284-96.
36. Benzoubir N, Lejamtel C, Battaglia S, et al. HCV core-mediated activation of latent TGF-β via thrombospondin drives the crosstalk between hepatocytes and stromal environment. *J Hepatol* 2013;59(6):1160-8.
37. Nakagawa H, Maeda S, Yoshida H, et al. Serum IL-6 levels and the risk for hepatocarcinogenesis in chronic hepatitis C patients: an analysis based on gender differences. *Int J Cancer* 2009;125 (10):2264-9.

38. Elgonimy A, Farouk S, Abdel Rahman E.The pathogenesis of cytokines in preportal fibrosis of human infected with schistosomiasis and viral hepatitis. *Egypt J Hosp Med* 2005; 20(1): 16-28.
39. Abdeen Radwan A, Abd-Elazeem Hefney NE, Mohammed Kholef EF, Elebidi A, Mahmoud H. Transforming Growth Factor B as a Marker of Hepatocellular Carcinoma in Patients with Chronic Hepatitis C Virus Infection. *Rep Biochem Mol Biol* 2023;11(4):702-9.
40. Elbanan WK, Fathy SA, Ibrahim RA, Hegazy MGA. Assessment of interleukin 17 and transforming growth factor-beta 1 in hepatitis C patients with disease progression. *Trop Biomed* 2020;37(4):1093-104.