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Assessment of interleukin 17 and transforming growth factor-beta 1 in hepatitis C patients with disease progression

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Abstract. Hepatitis C virus (HCV) infection in Egypt is the most serious health problem. Identifying HCV-positive persons at high risk of early complications can help prioritize treatment decisions. Recently, attention has been directed to non-invasive, accurate alternatives using serum biochemical markers. The transforming growth factor β 1/interleukins pathway plays an important role in the process of cell injury and inflammation. Thus, TGF-β1 and IL-17 were assessed in serum of chronic HCV patients with correlation to hepatic inflammatory and fibrotic status. The quantitative serum levels of TGF-β1 and IL-17 were analyzed among chronic hepatitis C (CHC) patients (n=75) and normal control (NC) subjects (n=15). Disease severity in patients was assessed using the Child-Pugh scores and METAVIR. Serum levels of TGF-β1 and IL-17 were significantly increased in HCV patients compared to control group. Furthermore, the levels of TGF-β1 and Il-17 were positively correlated to serum transaminases and alpha-fetoprotein and they were negatively correlated with serum albumin and platelets. Additionally, the serum levels of TGF-β1 and Il-17 were associated with inflammation grades and stages of liver fibrosis. TGF-β1 and IL-17 may be hopeful serum biomarkers concerned in the progression of liver inflammation and fibrosis accompanying chronic HCV infection. Therefore, they could be used in the future as targets for anti-fibrotic therapy of chronic HCV to ameliorate the disease progress.

INTRODUCTION

Hepatitis C virus (HCV) infection is a major public health burden in Egypt, where it bears the highest prevalence rate in the world (Gomaa *et al.*, 2017). HCV-infected people are at high risk for developing chronic liver disease, cirrhosis, and hepatocellular carcinoma (HCC). HCV accounts for about 27% of cirrhotic cases and about 25% of HCC cases worldwide (Shepard *et al.*, 2005).

This RNA virus does not affect the liver uniformly even with adequately sized biopsies, and cirrhosis maybe missed in 15–30% of liver biopsies (Brenner, 2009). This arouses the urgent need to find new biomarkers for diagnosis, treatment, and prognosis of HCV. During the past decade, much of the accumulated evidence supported the role of interleukins and growth factors in linking inflammation, fibrosis, and tumorigenesis (Mitchell *et al.*, 2008).

Cirrhosis is a diffuse process of hepatic fibrosis and regenerative nodular formation of unknown pathogenesis. Hepatic fibrosis is the main hallmark of chronic liver disease. It is a complex process that involves the activation of extracellular matrix synthesis, cytokine release, and tissue remodeling (Border & Noble, 1994; Bissell & Maher, 1996).

Transforming growth factor β1 (TGF-β1) have been suggested to be involved in the pathogenesis of fibrosis in chronic hepatitis C (Kotsiri *et al.*, 2016). It stimulates the synthesis of extracellular matrix proteins and their receptors, and inhibits synthesis of matrix-degrading proteolytic enzymes, resulting in the formation of fibrosis and tissue repair (Border & Noble, 1994).

IL-17 is a powerful chemoattractant for neutrophils and has been reported to be involved in many immune processes, most notably in inducing and mediating proinflammatory responses e.g. several autoimmune diseases, allergic diseases, asthma and pulmonary infection (Woltman *et al.*, 2000; Toda *et al.*, 2003). Several studies have reported that the frequency of IL-17 cells is increased in the portal areas of livers from chronic HCV infected patients (Harada *et al.*, 2009).

Several groups of investigators recognized the role of IL-17 in hepatitis B and its sequelae (Wang *et al.*, 2011; Du *et al.*, 2013). However, there has been limited data of the role of IL-17 in HCV infected patients. In addition, Egyptian studies on the role of IL-17 in the immunopathogenesis of HCV are limited.

In patients with chronic liver disease, it is critical to have a clear understanding of hepatic fibrosis and its severity because it could help clinicians predict patient prognosis. Therefore, the aims of this study were to assess serum TGF-β1 and IL-17 levels in HCV-positive patients with chronic liver disease and their relation to the severity of liver disease.

MATERIALS AND METHODS

Patients

The cross-sectional study protocol was performed in accordance with the Ethics Committee of the Faculty of Science, Ain shams University, Egypt, during the period from April 2015 to April 2017. Informed written consent was obtained from all subjects included in this study. Ninety subjects were included in the study; they were divided into 2 groups:

- Chronic hepatitis C (CHC) group: included 75 hepatitis C virus infected patients recruited from Medical Insurance Hospitals. Diagnosis of CHC was done by assays for HCV antibody testing, by using of a commercial kite (Ortho clinical diagnostic; USA) and confirmed by real-time detection system (Applied Biosystem StepOne TM Real Time PCR system Thermal Cycler Block, Singapore) HCV RNA PCR (more than 50 IU/ml).

- Control group: included 15 healthy control subjects matched age and sex.

All participants were subjected to full medical history, thorough clinical examination, laboratory investigation, abdominal ultrasonography, and ultrasonography guided needle liver biopsy.

Exclusion criteria included the following: coinfections with hepatitis B virus (HBV) or HIV, HCC, organ transplantation, immunosuppression, autoimmune disease, diabetes, Schistosoma, and other malignant comorbidities. Also, patients received antiviral therapy, and chemotherapy was excluded.

Regarding the histopathological data, stages of brosis and grades of inammation were determined. The Child-Pugh scoring system (inflammation grades) was performed for all patients using two clinical variables, ascites and hepatic encephalopathy, and three laboratory parameters, serum total bilirubin, albumin levels, and prothrombin time (PT) from one to three to a maximum score of 15 (Ishak *et al.*, 1995). Based on these the patients were assigned to one of three classes A, B and C, with Child-Pugh scores 5-6, 7-9 and 10-15, respectively (Pugh *et al.*, 1973). Fibrosis was staged on a four-point scale according to METAVIR (F0 indicated no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis and few septa; F3, numerous septa without cirrhosis; F4, cirrhosis).

Detailed demographic, clinical and biochemical characteristics of the patients are presented in Table 1.

METHODS

Specimen collection and processing

Five milliliters of blood were withdrawn from each patient under complete aseptic conditions. Sera were separated and stored frozen at -70°C until analysis.

Routine Investigations

Liver profiles – aspartate transaminase (AST) and alanine transaminase (ALT), serum albumin (Alb), total bilirubin (TBil), and other biochemical indices were determined with an automatic biochemical analyzer (LX-20; Beckman, USA). Complete blood count (CBC) was determined by automated cell counter (ERMA Inc., Tokyo, Model PCE-210). The PT and INR were measured with an automated coagulation analyzer (IL TOP700; Werfen Group, San Jose, CA, USA). Álpha-fetoprotein (AFP) was performed by using Monobind INC kit, USA.

ELISA Biomarkers

The concentrations of interleukin 17 (IL-17) and transforming growth factor beta 1 (TGF-β1) were determined by enzyme-linked immunosorbent assay (ELISA) according to manufacturer's instructions (SunRed; hepatology company; Shangahai, China). Absorbance was measured at 450nm against blank using an ELISA reader (Ray Biotech, Canada).

Data Analysis

The data was collected and entered into the personal computer. The resulting values are expressed as mean ± standard deviation and the statistical analysis was done using Statistical Package for Social Sciences; SPSS software version 17 for Windows (SPSS Inc., Chicago, IL, USA). The p values <0.05 were considered statistically significant. The Kruskal Wallis and Mann-Whitney U tests were used to compare the continuous variables. Numerical data were analyzed by the $\chi 2$ test, and quantitative differences between groups were processed by analysis of variance. Spearman's rank correlation was performed between variables. To confirm the effectiveness of TGF-β1 and IL-17, the receiver operating characteristic (ROC) curve was used to determine the area under the ROC curve. The cutoff value was set up to find the sensitivity and specificity as well as the predictability for positives and negatives. For ROC analysis, an area under the curve (AUC) of 1.0 indicates perfect discrimination

RESULTS

The baseline demographic and biochemical characteristics of the study population were summarized in Table 1. The study included 75 HCV infected patients; 63 males and 12

Table 1. Demographic and Clinicopathologic characteristics of the studied groups

Parameter	Control group (n=15)	CHC group (n=75)
Age (years)	40.22 ± 9.765[a]	49.10 ± 8.582[b]
Gender m/f (%)	10/5	63/12
Albumin (g/dl)	4.178 ± 0.517[a]	3.435 ± 0.492[b]
INR	0.961 ± 0.119[a]	1.199 ± 0.226[b]
T. bilirubin (mg/dl)	0.583 ± 0.188[a]	1.084 ± 0.618[b]
AST (IU/ml)	17.39 ± 7.875[a]	49.14 ± 27.055[b]
ALT (IU/ml)	19.33 ± 7.316[a]	61.50 ± 41.25[b]
Platelets (10^3/5l)	304.17 ± 38.912[a]	203.50 ± 66.60[b]

Values are expressed as means ± S.D.; mean values within a row not sharing a common superscript letter (a,b) were significantly different; $P < 0.05$.

females with mean age of 49.1 ± 8.58 years. Control group included 10 females and 5 males with a mean age of 40.22 ± 9.76 years. The serum TGF-β1 concentration was higher in patients with HCV than in the control group (Table 2). According to the Child-Pugh criteria, significant differences in concentrations of this element between the control group and groups with stages B and C of liver cirrhosis was observed. However, no statistically significant differences in the concentrations of TGF-β1 were demonstrated when comparing control with group A of liver cirrhosis. Post-hoc tests revealed significant difference in TGF-β1 concentration when compared group B with group C of liver cirrhosis. Regarding the relation with the stages of brosis, TGF-beta serum levels showed a signicant dierence ($P<0.001$) between grades of inammation as well as all stages of brosis except between F1 and F2 ($P>0.05$) (Table 3).

The highest concentration of interleukin-17 was observed in patients with liver fibrosis in stage C and B, whereas the lowest one in the control group. Multiple comparison tests revealed signicant differences between control group as compared to groups with various stages of severity of liver fibrosis – A, B and C. In addition, a statistically signicant difference between patients with stage A, B and C according to Child-Pugh was observed (Table 2). Moreover, a signicant dierence was found between patients with dierent stages of brosis (F0-F1, F2 and F3-F4) ($P<0.001$) see Table 3.

α-fetoprotein concentration showed significant difference when comparing control with stage B and C liver fibrosis, while a non-significant difference was observed when compared with group A liver fibrosis (Table 2). Furthermore, a signicant difference ($P<0.001$) between control and the late stages of brosis was seen (F3, F4) (Table 3).

Regarding correlation of TGF-β & IL-17 with the measured variables, negative correlation was observed with albumin concentration and platelets count. While positive correlation of TGF-β & IL-17 was found with 5-Fetoprotein; AST; ALT, total bilirubin and international normalised ratio (INR) (Table 4).

ROC curve was designed for discriminating HCV patients from control group (Figure 1A), and results revealed that TGF-β1, IL-17 and AFP had sensitivity of 90, 88 and 76% respectively, specificity of 77.8, 77.8 and 83.3% respectively, at cutoff values of 56.2, 1.24 and 4.15 respectively (Table 5). Moreover, combined sensitivity and specificity of TGF-β1, IL-17 and AFP was estimated as shown in Table 6. It showed the highest improved sensitivity (92%) and specificity (83.3%) even in early stage of fibrosis when combined TGF-β1 with IL-17.

Furthermore, ROC discrimination analysis of control from early fibrosis degree (F1/F2), showed the same cut-off values for TGF-β1, IL-17 and AFP as previously mentioned with the same specificities and decreased sensitivities to reach 85.3%, 82.4% and 64.7% respectively (Table 5, Figure 1B). Similarly, for the discrimination of control from advanced fibrosis patients (F3/F4), the cut-off values of TGF-β1, IL-17 and AFP were 202.5 pg/ml, 3.35 pg/ml and 6.2 ng/ml respectively with 100% sensitivity for both TGF-β1 and IL-17, and 93.8% for AFP, also specificity was 100% for all (Table 5 and Figure 1C).

In the discrimination analysis of early fibrosis (F1/F2) from advanced fibrosis (F3/F4), the cut-off values of TGF-β1, IL-17 and AFP were 330.5 pg/ml, 5.35 pg/ml and 8.55 ng/mL respectively with sensitivities 93.8%, 93.8% and 87.5% respectively; while specificities were 97.1%, 91.2% and 82.4% respectively (Table 5, Figure 1D).

DISCUSSION

Globally, HCV is considered as one of the major causes of chronic liver diseases, which include inammation, brosis and cirrhosis. Furthermore, HCV leads to increased morbidity and mortality in hepatocellular carcinoma (Liu *et al.*, 2007). HCV is currently the most substantial public health problem in Egypt.

Table 2. Relation between inflammation grades (Child-Pugh classification) and evaluated biomarkers in the studied groups

Parameter	Controls (n=15)	HCV patients (n=75)	HCV (n=75)		
			A (score 5-6) (n=31)	B (score 7-9) (n=24)	C (score 10-15) (n=20)
TGF-β1 (pg/ml)	55.82 ± 26.44[a]	353.35 ± 376.47[b]	127.97 ± 79.37[a]	639.46 ± 355.40[b]	1051.80 ± 93.41[c]
IL-17 (pg/ml)	1.14 ± .218[a]	5.56 ± 4.860[b]	2.47 ± 1.57[b]	9.92 ± 4.35[c]	13.40 ± 1.83[d]
AFP (ng/ml)	3.64 ± .653[a]	9.78 ± 10.203[b]	4.91 ± 2.71[a]	14.23 ± 10.86[b]	28.20 ± 12.72[c]

Values are expressed as means ± S.D.; mean values within a row not sharing a common superscript letter (a,b,c) were significantly different; $P < 0.05$.

Table 3. Relation between stages of brosis and evaluated biomarkers in the studied groups

Parameter	Controls (n=15)	HCV patients (n=75)	HCV (n= 75)			
			F0-F1 (n=20)	F2 (n=23)	F3 (n=22)	F4 (n=10)
TGF-β1 (pg/ml)	55.82 ± 26.44[a]	353.35 ± 376.47[b]	62.83 ± 27.28[a]	172.55 ± 90.07[a]	704.45 ± 352.76[b]	1051.80 ± 99.07[c]
IL-17 (pg/ml)	1.14 ± .218[a]	5.56 ± 4.860[b]	1.281 ± 0.39[a]	3.461 ± 1.75[b]	10.518 ± 4.07[c]	13.74 ± 1.93[d]
AFP (ng/ml)	3.64 ± .653[a]	9.78 ± 10.203[b]	3.827 ± 1.82[a]	6.360 ± 3.55[a]	14.472 ± 12.03[b]	28.280 ± 13.49[c]

Values are expressed as means ± S.D.; mean values within a row not sharing a common superscript letter (a,b,c,d) were significantly different; $P < 0.05$.

Table 4. Spearman's correlation between TGF-β1 and IL-17 and other variables in CHC patients

Study parameters	TGF-β		IL-17	
	r	*p*	r	*p*
AFP	0.735	<0.001	0.731	<0.001
AST	0.645	<0.001	0.685	<0.001
ALT	0.668	<0.001	0.666	<0.001
Alb	-0.691	<0.001	-0.668	<0.001
TBIL	0.680	<0.001	0.701	<0.001
HGB	-0.190	NS	-0.233	NS
PLT	-0.613	<0.001	-0.572	<0.001
INR	0.630	<0.001	0.608	<0.001

Correlation is highly significant at $p < 0.01$, NS is non-significant.

Table 5. Area under curve, cut off value and performance characteristics of AFP, TGF-β1 and IL-17 to discriminating control from HCV group, control from early fibrosis group, control from advanced fibrosis group, early from advanced fibrosis group

Parameter	AUC	Cutoff value	Sensitivity	Specificity
Control vs HCV				
TGF-β1 (pg/ml)	0.913*	56.2	90%	77.8%
IL-17 (pg/ml)	0.918*	1.24	88%	77.8%
AFP (ng/ml)	0.788*	4.15	76%	83.3%
Control vs Early				
TGF-β1 (pg/ml)	0.873*	56.2	85.3%	77.8%
IL-17 (pg/ml)	0.880*	1.24	82.4%	77.8%
AFP (ng/ml)	0.690*	4.15	64.7%	83.3%
Control vs Advanced				
TGF-β1 (pg/ml)	1.0*	202.5	100%	100%
IL-17 (pg/ml)	1.0*	3.35	100%	100%
AFP (ng/ml)	0.998*	6.2	93.8%	100%
Early vs Advanced				
TGF-β1 (pg/ml)	0.985*	330.5	93.8%	97.1%
IL-17 (pg/ml)	0.978*	5.35	93.8%	91.2%
AFP (ng/ml)	0.924*	8.55	87.5%	82.4%

* $P < 0.05$ is significant.

Table 6. Combined sensitivity and specificity of all studied parameters to discriminate control from HCV group

Combined parameters	Combined Sensitivity	Combined Specificity
TGF-β1+AFP	96%	61.1%
IL-17 +AFP	92%	61.1%
TGF-β1+ IL-17	92%	83.3%
TGF-β1+ IL-17 +AFP	96%	66.7%

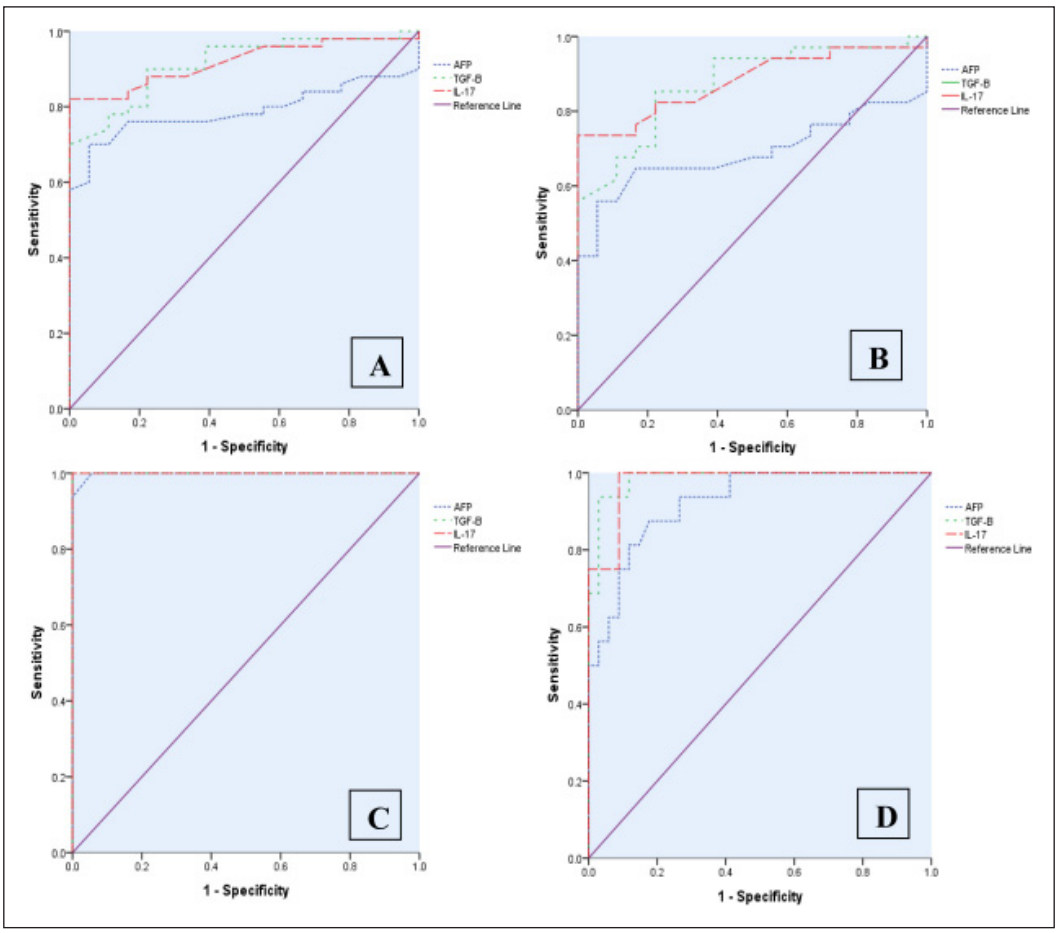

Figure 1. ROC curves for TGF-β1, IL-17 and AFP serum levels for discriminating (A) control from HCV group. (B) control from early fibrosis group (F1/F2). (C) control from advanced fibrosis group (F3/F4) (D) early fibrosis group (F1/F2) from advanced fibrosis group (F3/F4).

Interleukine-17 (IL-17) is the cytokine which is secreted by CD4+ Th17 cells and is considered to be pro-inflammatory and pro-fibrotic (Miossec *et al.*, 2009). Moreover, transforming growth factor beta-1 (TGF-β1) is another key cytokine that is positively implicated in the development of liver inammation and brosis. It participates in many important events leading to liver brosis, such as expression of other probrogenic intermediaries as cytokine connective tissue growth factor (CTGF) (Kirmaz *et al.*, 2004; Nagaraja *et al.*, 2012). Therefore; this study was performed to investigate the association of serum level of IL-17 and TGF-β1 with disease severity in HCV infected patients.

In the present study, the role of IL-17 in HCV-induced liver brosis was examined. Its serum concentrations in HCV patients were signicantly higher compared to the control group; this was verified by many others (Fathy *et al.*, 2011; Ghazy *et al.*, 2012).

Previous studies have reported on a close correlation between virus-induced liver inflammation, infiltration in chronic hepatitis c infections and activation of Th17 cells and the amount of liver damage caused by the antiviral immune response (Lemmers *et al.*, 2009; Chang *et al.*, 2012). Our study demonstrated positive relation between IL-17 and child score A, B and C with the highest level in C class. In agreement with previous reports (Ghazy *et al.*, 2012; Shi

et al., 2015; Kassim *et al.*, 2017), IL-17 has critical role in the pathogenesis of liver fibrosis. IL-17 produced by neutrophils and CD4+ and CD8+ T cells promoted proinflammatory cytokine expression, neutrophil influx, liver injury, inflammation, and fibrosis through hepatic stellate cell activation. There is an increase in the level of IL-17 with increasing inflammation, fibrosis and cirrhosis (Tan *et al.*, 2013). While Hassan *et al.* (2014) reported no correlation between child score and IL-17 level.

In the current study, serum levels of IL-17 were significantly associated with different stages of fibrosis (F1; F2; F3; F4) and there were previous studies that support this nding (Tan *et al.*, 2013; Hassan *et al.*, 2014). The relation of IL-17 to liver fibrosis remains elusive. Liver fibrosis is a severe, life-threatening clinical condition resulting from non-resolving hepatitis of different origins. IL-17 might have a pro-fibrogenic effect through independent mechanisms (Harada *et al.*, 2009; Du *et al.*, 2013). IL-17 excites Kuper cells to prompt several inflammatory cytokines including the major fibrogenic cytokine TGF-β1; which in turn, induce activation of the hepatic stellate cells (HSC) into myobroblasts. Additionally, IL-17 directly induce HSCs to express collagen type I consequently, encouraging their activation into fibrogenic myobroblasts via the signal transducer and activator of transcription 3 (Stat 3) signaling pathway. Moreover, it has been described elsewhere that intrahepatic IL-17 expression was powerfully associated with the serum indices of hepatic brosis, which is well thought-out as a vital pathological process in the development of liver cirrhosis (Woltman *et al.*, 2000).

Astonishingly, Lemmers *et al.* (2009) stated that when relating the serum levels of IL-17 with the different stages of fibrosis, no significant difference was detected. However, one could assume that if Th17 is implicated in chronic HCV inflammation, the hallmark cytokine would be associated with hepatic fibrosis, and it would be a potential hepatic fibrosis biomarker in cases of chronic HCV infection. Still, a study conducted on a large cohort of patients with varying degrees of fibrosis; was unable to find any significant association between fibrotic scores and serum IL-17 in chronic HCV patients (Lemmers *et al.*, 2009). This was with agreement of another study which found even an inverse correlation of liver fibrosis stage with HCV-specific IL-17 (Li *et al.*, 2012).

This higher serum level of IL-17 in patients with chronic liver disease goes with what has been reported previously that serum IL-17 level was increased in liver injuries following chronic hepatitis and cirrhosis supporting a role for IL-17 as a chronic disease inducer in the pathogenesis and/or progression of liver fibrosis (Chang *et al.*, 2012).

The correlation between IL-17 serum levels and the inflammation grades of these patients was studied. A significant difference between the groups of inflammation was detected, which confirmed that IL17 played an important role in regulation of inflammation and this was in concordance with other studies (Tan *et al.*, 2013).

Alfa fetoprotein was positively correlated with IL-17 level ($p<0.05$). Which is compatible with results of Ghazy *et al.* (2012) and not compatible with that reported by Liao *et al.* (2013).

We reported significant correlations between the serum concentration of IL-17 and that of some indicators of liver function e.g. ALT, AST and serum albumin suggesting that IL17 is, to a certain amount, associated with the degree of liver damage. Moreover, this significant correlation between serum IL-17 and ALT was in accordance with several studies which showed that IL-17 level correlated directly with severity of liver inflammation (Harada *et al.*, 2009; Wang *et al.*, 2011). This is possibly because IL-17 stimulates a variety of immune cells to release inflammatory mediators, leading to frequent inflammation of the liver and decline of liver function (Harada *et al.*, 2009). Nevertheless, Du *et al.* (2013) disclosed in his earlier report no correlation between serum IL-17 and ALT where the latter can be easily affected by drugs which decrease its level (Woltman *et al.*, 2000). In our study, serum concentrations of IL-17 rose

significantly, which were negatively correlated with albumin and platelet count, but positively correlated with TBIL, ALT and AST.

Transforming growth factor beta-1 (TGF-β1) is another key cytokine that is positively implicated in the development of liver inflammation and fibrosis. Several lines of evidence point to participation of TGF-β1 in many important events leading to liver fibrosis, such as expression of other profibrogenic intermediaries (Kirmaz *et al.*, 2004; Nagaraja *et al.*, 2012).

In this study, we have investigated TGF-β1 serum concentrations in HCV patients with relation to degrees of liver inflammation and fibrosis. TGF-β1 serum level was significantly higher in HCV patients than in control group. Moreover, the higher the frequency of raised TGF-β1 level, the worse the disease activity of chronic liver disease (Table 2), this was evidenced also in many previous studies as Kamal *et al.* (2006).

Obviously, hepatic fibrosis is a frequent complication of chronic HCV infection. It was found in a study on the recipients of bone marrow transplants that raised circulating TGF-β1 levels are highly predictive of the development of hepatic fibrosis (Anscher *et al.*, 1993). Likewise, Clemente *et al.* (2006) and Gressner *et al.* (2002) reported that the increased serum levels of TGF-β1 in HCV-related chronic liver disease were significantly associated with different stages of fibrosis which was in consistence with our study specifically stages (F2; F3; F4) but with no difference between lower stages (F1; F2). That may be attributed to the small sample size within lower stages (Table 3).

In the current study, we found that the increased serum levels of TGF-β1 are in parallel with the severity of liver fibrosis in the patient groups. Similar results have been reported by Clemente *et al.* (2006). This finding is signifying that locally released TGF-β1 could be in charge for upregulation of fibrogentic cytokines observed in patients with HCV related chronic liver disease.

In this study, we have examined TGF-β1 serum levels in HCV patients in relative to child score (A, B and C), where we found significant elevation in relation to grades of inflammation as reported by Kamal *et al.* (2006). Moreover, the higher the frequency of raised TGF-β1 level, the worse the disease activity of chronic liver disease (Table 2).

These remarks supported the perception that chronic frequent injury might result in a sustained increase in TGF-β1 level, leading to the progressive deposition of extracellular matrix and tissue fibrosis (Bissell and Maher, 1996; Border and Noble, 1994).

TGF-β1 is strongly related to IL-17, TGF-β1 is considered to have unique capacity to direct T cell lineage commitment to pro-inflammatory Th17 (Wahl, 2007).

Presser *et al.* (2013), revealed the molecular mechanism of TGF-β gene expression in reaction to HCV infection. They verified that HCV-induced transcription factors such as NF-kB and STAT-3 were involved in TGF-β gene expression. Similarly, HCV induced TGF-β1 gene expression was mediated via the activation of certain cellular kinases such as p38 MAPK, JNK, and MEK1/2. Additionally, Chusri *et al.* (2016) reported that TGF-β1, play striking roles in liver fibrogenesis through the generation of different reactive oxygen species (ROS).

The correlation between more severe liver damage and the higher level of TGF-β1 in patients with HCV infection indicate that the TGF-β1 level correlated with disease activity. This opinion might be supported by the association between urinary TGF-β1 levels and diminished liver function (Table 1). Thus, our data strongly indicated that a raised TGF-β1 level was an indicator of more severe liver damage.

In our study, we found that TGF-β1 were negatively correlated with ALB and platelet counts and positively correlated with AFP, ALT, AST, Total Bilirubin and INR suggesting that TGF-β1 may reflect the extent of liver injury.

ROC analysis has been recommended to calculate the power of the assays to detect advanced liver fibrosis (Zheng *et al.*, 2002). In this study, the ability of examined biochemical markers (IL-17, TGF-β1 and AFP) to differentiate patients with liver fibrosis from controls was significant. The

best discriminating values of IL-17, TGF-β1 and AFP which gave the highest sensitivities and specificities were, 56.2 pg/ml for TGF-β1 with sensitivity 90% and specificity 77.8%, 1.24 pg/ml for IL-17 with sensitivity 88% and specificity 77.8%, 4.15 ng/ml for AFP with sensitivity 76% and specificity 83.3% (Figure 1, Table 5).

When a marker is used for workup of liver fibrosis and inflammation, it should have a high sensitivity. Accordingly, the combined use of studied parameters was superior to the use of them independently (Table 6). Moreover, both the sensitivity and the specificity were improved when combined TGF-β1 with IL-17.

On the other hand, since chronic HCV infection is correlated with inflammation and fibrosis of the liver, a non-invasive biomarker is essential to grade the disease stage and to start therapy in order to prevent progression to HCC. Thus, the current studied parameters were evaluated as discrimination markers between different liver fibrosis stages. We demonstrated that serum TGF-β1 with IL-17 concentration positively and significantly associates with the stage of fibrosis in patients with HCV infection which could be utilized as a biomarker as well as a prognostic index towards HCC. The study also showed that serum TGF-β1 with IL-17 levels reveal the extent of hepatic fibrosis and could be used as a non-invasive biomarker to evaluate the grade of fibrosis in HCV patients that would help to reduce the number of liver biopsies.

Many blood tests have been suggested as alternatives to liver biopsy for detecting the degree of fibrosis or cirrhosis. Amongst, aspartate aminotransferase-platelet ratio index (APRI) and Fibro-Test have been confirmed for patients with HCV infection (Chou & Wasson, 2013). In the existing study also, we calculated APRI, but observed that the discriminative ability of APRI was poor compared to serum TGF-β1 with IL-17.

In conclusion, the attained outcomes highlighted the possible clinical value of synergetic association of IL-17 and TGF-β1 in help determining the rate at which hepatic fibrosis is developing or relapsing. TGF-β1 and IL-17 levels were increased with increasing liver disease progression. Thus, may be an important biological marker for the immunopathogenesis of chronic hepatitis and liver fibrosis. Thus, blocking of TGF-β1 and IL-17 expression may be a potential target for controlling the inflammatory response in chronic hepatitis and liver fibrosis.

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Conflict of Interest

All the authors declare no conflict of interest. This research received no specific grant from any funding agency in the public or commercial sphere.

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