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Transforming Growth Factor B as a Marker of Hepatocellular Carcinoma in Patients with Chronic Hepatitis C Virus Infection

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Abstract

Background: The Transforming Growth Factor-beta (TGF-β) is one of the main growth factors associated with fibrosis or cirrhosis progression in the liver, but its role in hepatocarcinogenesis is controversial. To highlight the role of Transforming Growth Factor β as a marker of Hepatocellular carcinoma (HCC) in patients with chronic hepatitis C virus (HCV) infection.

Methods: Ninety subjects were enrolled in this study, classified into three groups: Group I (chronic HCV group) included 30 patients with chronic HCV infection; Group II (HCC group) include 30 patients having HCC and chronic HCV infection and Group III consisted of 30 age and sex-matched healthy controls. TGF-β was evaluated in all the enrollees and its levels were correlated to liver function and other clinical parameters.

Results: TGF-β was found significantly higher in HCC group than in control and chronic HCV ($P<0.001$). In addition, it was correlated with biochemical and clinical parameters of cancer.

Conclusions: Patients with HCC showed increased level of TGF-β compared to chronic HCV infection patients and controls.

Keywords: Chronic HCV, Liver cirrhosis, Transforming Growth Factor β.

Introduction

Hepatocellular carcinoma (HCC) is considered the fifth most common cancer in men and the ninth in women and represents an urgent clinical problem, being the second leading cause of cancer-related death worldwide (1, 2).

Hepatitis C virus (HCV) infection is a major risk factor for chronic liver disease and for the increasing HCC incidence. Approximately 3% of the world population is infected with HCV, and the severe consequences of virus infection makes HCV one of the most pressing emergencies worldwide (3,4) Most infected patients develop a chronic HCV, characterized by inflammation-induced lesions in the liver frequently associated with steatohepatitis and progressive fibrosis which evolves in cirrhosis in about 10 to20% or HCC in 1-5% of the patients (5). In chronic HCV people, the risk of developing HCC is strictly correlated to fibrosis stage, with the incidence of HCC more frequent in patients with cirrhotic liver than in those with mild fibrosis (6). Moreover, several risk factors, such as hepatitis B virus or HIV co-infection, obesity, insulin resistance or nonalcoholic steatohepatitis actively enhance HCV-related HCC progression (7).

Transforming growth factor β (TGF-β) superfamily is known to be involved in

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embryonic development, adult tissue homeostasis, and disease pathogenesis. Specifically, it has been shown to control proliferation, differentiation, apoptosis, migration, extracellular matrix remodeling, immune functions, and tumor invasion/ metastasis (8).

TGF-β enhances hepatic stellate cell activation, stimulates collagen gene transcription, and suppresses matrix metalloproteinase expression. Thus, TGF-β, as well as its intracellular mediators; SMAD proteins, can be potential therapeutic targets for liver fibrosis. TGF-β inhibits hepatocyte proliferation, but it also can lead to HCC promotion. TGF-β has been shown to play both tumor-suppressive at early stage and tumor promoting roles at later stage (9). At the early stage of tumor genesis, TGF-β1 inhibited normal cell growth and tumor genesis by suppressing G1/S phase transition, in later stages; malignant cells become resistant to suppressive effects of TGF-β either through mutation and/or functional inactivation of TGF-β receptors or by downstream alterations in the SMAD signaling pathway (10, 11). Mutations in downstream TGF-β signaling components cause variable attenuations or complete loss of expression; these mutations, which have been detected in many common tumors, affect TGF-β signal transmission that potentially results in human cancer development and progression (12). TGF-β1 expression was related to tumor grade and pathological stage. Furthermore, overexpression of plasma TGF-β1 was associated with invasiveness of HCC and worse prognosis (13)

The present study aimed to high light the role of Transforming Growth Factor β as a marker of hepatocellular carcinoma in patients with chronic hepatitis C virus infection.

Materials and Methods

The study was approved by the Ethics Unit, Faculty of Medicine, Aswan University, Aswan, Egypt, and an informed written consent was obtained from all the participants of the study.

Selection Criteria

Patients chronic HCV infection and various stages of liver fibrosis and patients with HCC and chronic HCV infection. The patients were classified into three groups as following:

Group I (Chronic HVC) consisted of 30 patients with chronic HCV infection. Diagnosis of chronic HCV was based on persistence of HCV antibodies and HCV RNA in the serum of the patients for more than 6 months. Cirrhosis was diagnosed with liver fibroscan, abdominal sonography and biochemical evidence of parenchymal damage with or without endoscopic evidence of esophageal or gastric varices (14)

Group II (HCC group) consisted of 30 patients with HCC and chronic HCV infection. The HCC diagnostic criteria were based on the guidelines proposed by European Association for the Study of the Liver (EASL) (15)

Group III consisted of 30 age-matched and sex-matched healthy controls.

The patients with Extra hepatic malignancy, pregnancy, inadequately controlled diabetes mellitus, Co-infection with hepatitis B virus and Co-infection with HIV were excluded from the study.

Clinical investigations

All subjects were subjected to detailed history taking, including, age, sex, and drug use, full clinical examination including, measurements of body mass index, vital signs, abdominal examination, and other systems examination.

Abdominal ultrasound was done to investigate the liver cirrhosis with examination of liver size, echogenicity, hepatic focal lesion, splenic size, portal vein diameter, and presence of ascites**.** The severity of liver disease was estimated by Child-Pugh and MELD scores (16) calculated based on laboratory tests performed on admission.

Laboratory tests

Laboratory investigations were including complete blood picture, Erythrocyte sedimentation rate, fibrinogen, ALT (alanine transaminase), AST (aspartate transaminase), ALP (alkaline Phosphatase), GGT (gamma

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glutamyl transaminase), HbsAg and HCV-Ab for all patients as routine hospital tests. Serum creatinine, CRP (C-reactive protein), serum albumin, serum bilirubin (total and direct) were also measured using Cobas C analyzer. AFP (alpha fetoprotein) and TGF-β1 level assessment in serum using Human alpha Fetoprotein ELISA Kit (ab193765) and Human TGF beta 1 ELISA Kit (ab100647) ELISA kits, respectively.

Statistical analysis

Analysis of data was done using Statistical Package for the Social Science version 20 (SPSS Inc, Chicago, IL, USA). Quantitative variables were described as mean and standard deviation and range. Qualitative variables were described as number and percent. To compare parametric quantitative variables between the groups, One-way ANOVA was performed. Qualitative variables were compared using chi-square (X^2) test or Fisher's exact test when frequencies were below five. Pearson correlation coefficients were used to assess the association between two normally distributed variables. p- value< 0.05 is considered significant.

Results

Sixty patients enrolled from the internal medicine department of Aswan University Hospitals (Table 1). The patients were divided into two groups; Group I (Chronic HVC) consisted of 30 patients who had chronic HCV infection; Group II (HCC group) consisted of 30 patients already diagnosed as having HCC and had chronic HCV infection. In addition, 30 age-matched and sex-matched healthy controls were included in the study (Group III).

Table 1. Demographic data in between the studied groups.

Groups Parameters	**Control group (n=30)**		**Chronic HVC (n=30)**		**HCC (n=30)**		**P- value**
Age (years)							
Mean ± SD	54.720 ± 7.840		55.280 ± 7.003		55.345 ±4.043		P1=0.996 P2=0.543 P3=0.632
Range	(22-62)		(28-68)		(30-68)		
	No.	**%**	**No.**	**%**	**No.**	**%**	
Gender							
Female	17	56.7	13	43.3	15	50.0	P1=0.449 P2=0.442 P3=0.543
Male	13	43.3	17	56.7	15	50.0	

P1: between group I and II, P2: between group I and III, P3: between group II and III.

There was a significant difference in between the studied groups (control, Chronic HVC and HCC group) as regard to AST, ALT, ALP, T. bilirubin, D. bilirubin and CRP (Table 2).

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Table 2. Laboratory findings among the studied groups.

Parameter		Control Group (N = 30)	Chronic HVC group (N=30)	HCC group (N=30)	P
AST (U/L)	M ± SD	25.0 ± 5.7	46.2 ± 9.0	69.2 ± 5.8	P1< 0.001* P2< 0.001* P3< 0.001*
	Range	11.0 -33.0	26.0 - 108.0	36.0 - 118.0	
ALT (U/L)	M ± SD	27.0 ± 5.7	42.5 ± 9.2	64.1 ± 92.3	P1< 0.001* P2= 0.031* P3< 0.001*
	Range	8.0 -35.0	76.0 - 404.0	84.0 - 412.0	
ALP (U/L)	M ± SD	57.0 ± 3.6	173.6 ± 48.0	179.4 ± 41.8	P1< 0.001* P2=0.032 P3< 0.001*
	Range	23.0 -100.0	108.0 - 313.0	122.0 - 301.0	
T. bilirubin (mg/dL)	Mean ± SD	0.93 ± 0.2	1.1 ± 0.8	1.3 ± 0.4	P1=0.009* P2=0.043 P3=0.003
	Range	0.6 -1.2	0.6 - 3.6	0.6 - 2.5	
D. bilirubin (mg/dL)	Mean ± SD	0.1 ± 0.1	0.4 ± 0.5	0.5 ± 0.3	P1=0.003* P2=0.005 P3=0.006
	Range	0.1 - 0.3	0.1- 2.1	0.1 - 1.8	
CRP (mg/dL)	Mean ± SD	5.8 ± 0.5	44.8 ± 20.1	45.2 ± 20.4	P1=0.0067* P2=0.023 P3=0.005
	Range	0.5 -10	10.0 - 67.0	11.0 -78.0	
AFP (ng/ml)	M ± SD	1.71±1.34	33.3± 30.63	212.3± 209.11	P1< 0.001* P2< 0.001* P3< 0.001*
TGF-β (ng/ml)	M ± SD	8.56 ± 1.23	31.24 ± 11.43	221.66 ± 1.74	P1<0.001* P2<0.001* P3<0.001*

P1: between group I and II, P2: between group I and III, P3: between group II and III, *Significant.

There was a high significant difference in between the mean TGF-β level in control, Chronic HVC and in HCC group with P<0.001 (Table 2).

TGF-β had excellent Diagnostic performance with an area under curve (AUC) of 0.940 (95% CI = 0.840 to 1.040, p-value =0.001). A best cut-off criterion of TGF-β ≤ 116.78 could discriminate between patients with HCC from control with a sensitivity of 90.0% and specificity of 80% (Fig. 1).

There is a high significant correlation in between age, TGF-β level and ALT, AST, Serum albumin, T. bilirubin and CRP. There was a significant correlation in between TGF-β level and AFP, PT serum creatinine and urea (Table 3).

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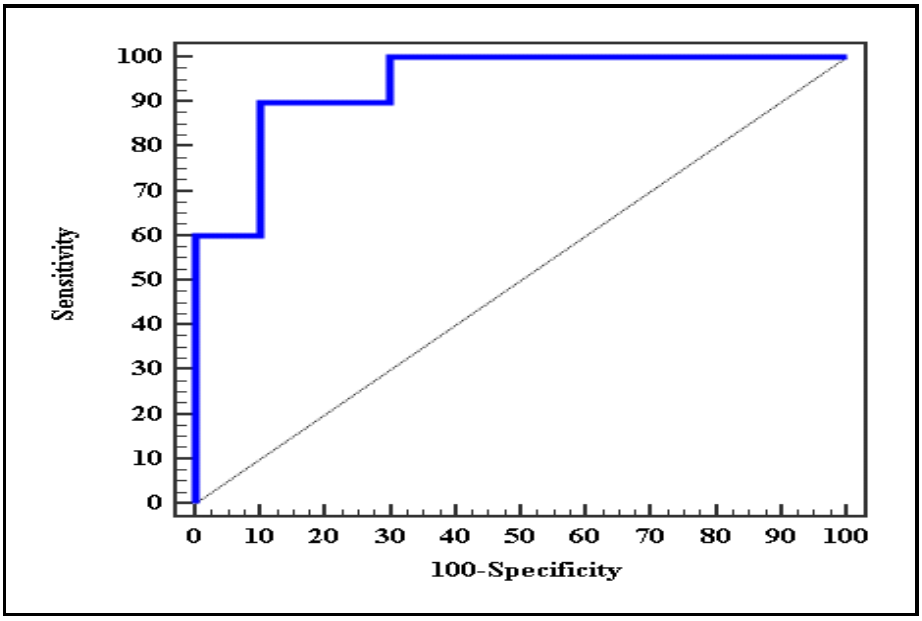

Fig. 1. ROC curve for TGF-β (ng/ml) in cirrhotic patients (AUC = o.940 ,95% CI = 0.840 to 1.040, p-value =0.001).

Table 3. Correlation between TGF-β and different parameters in HCC group.

Parameter	TGF-β	
	r	P
Age (years)	0.172	0.005
Gender	0.258	0.684
Cr (mg/dl)	0.812	0.041
Urea (mg/dl)	0.752	0.029
ALT(U/L)	0.602	<0.001*
AST (U/L)	0.668	<0.001*
Serum albumin (g/dL)	0.892	<0.001*
Hb (g/dL)	-0.242	0.062
WBC count	0.582	0.058
Platelet count	0.355	0.052
AFP	0.790	0.002
T. bilirubin	0. 762	<0.001*
CRP	0. 784	<0.001*
PT	-0.912	0.025
Child class	0.265	0.598

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Discussion

In the current study, there was a high significant elevation of the mean TGF-β level in HCC group than in control and chronic HCV patients, with P<0.001 and that coincide with Abdel Salam et al (17), as they found that there was highly statistically significant difference between controls and cirrhotic patients with HCC and between cirrhotic patients with and without HCC (P<0.001). This finding agreed with the study in (18), who documented that, the value of TGF- β1 mRNA expression in patients with HCC was significantly higher compared to that in healthy volunteers (P< 0.0001), and that the expression of TGF- β1 mRNA tended to be higher among patients with advancing histological aggressiveness. In general, the larger the tumor is, the higher the TGF- β1 mRNA level.

In the current study, there was a high significant elevation of the mean TGF-β level in HCC group than in control and chronic HCV patients, with P< 0.001 and that coincide with Abdel Salam et al (17), as they found that there was highly statistically significant difference between controls and cirrhotic patients with HCC and between cirrhotic patients with and without HCC (P< 0.001). This finding agreed with the study in (18), who documented that, the value of TGF- β1 mRNA expression in patients with HCC was significantly higher compared to that in healthy volunteers (P< 0.000 1), and that the expression of TGF- β1 mRNA tended to be higher among patients with advancing histological aggressiveness. In general, the larger the tumor is, the higher the TGF- β1 mRNA level.

This also in agreement with Baghdady et al. (19), who found that the serum level of TGF-β1 was 232.25±70.53 ng/ml in the HCC group and 42.16±13.34 ng/ml in chronic HCV group, and the mean in the control group was 13.92±7.73 ng/ml, showing a highly significant difference with respect to the three groups.

The serum level of TGF-β1 in chronic HCV was lower than that in the HCC group, a finding reported by many authors (20-24).

On the other hand, it was reported by Farid et al. (25) TGF-β1 gene expression showed significant change among the HCC, liver cirrhosis (LC), and control groups (P= 0.001). TGF-β1 gene expression in HCC patients was significantly lower than in LC patients (P= 0.042) and the control group (P= 0.001). In addition, TGF-β1 gene expression in LC patients was significantly lower than in the control group (P= 0.002). This difference in correlation may be due to the difference in the sample size which was smaller than our study (20 HCC, 20 LC and 20 healthy volunteers). found that the serum TGF-β1 level increased particularly, but insignificantly, in HCC associated with HCV infection (26) and another study reported no significant difference was elicited between the two groups (27).

In the present study, TGF-β had excellent Diagnostic performance in HCC patients with a sensitivity of 90.0% and specificity of 80% which relatively near to the results of another study, stated that the sensitivity and specificity of TGF-β in the prediction of HCC were (88.6% and 74%) respectively (18). A previous study showed the same specificity (75%) but lower sensitivity (65%) for TGF-β1 gene expression to distinguish HCC and LC and they concluded that the combination of TGFβ1 gene expression and AFP level could be better (25).

In this study, by binary logistic regression analysis, the significant predictors of bad outcome in patients with HCC were age, higher TGF-β level, Child paugh score and higher AFP levels which near to the results in the study done by (17) who found that by univariable binary logistic regression analysis revealed that, age> 58 years, creatinine level > 1.3 (mg/dl), serum albumin level< 2.5 (g/dl), ESR> 80, AFP≥ 41(ng/ml) and level of gene expression≥ 1.85 fold increase were significant risk factors for HCC. Multivariable binary logistic regression analysis showed that AFP≥ 41 (ng/ml) and level of gene expression≥ 1.85-fold increase were significant independent predictors of HCC. This was agreed with a study documented that, highest risk for development of HCC by binary

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logistic regression for prediction of HCC cases were age more than 58 years, hypoalbuminemia and increase level of AFP (28).

A previous study documented those age≥ 50 years correlated with increasing risk of HCC development by univariate analysis of β1 gene expression and HCC risk in Egyptian patients with chronic hepatitis C. Moreover, there was no significant association between TGF- β1 potential risk factors of HCC in cirrhotic patients (29). Also, the decreased serum albumin levels remained significantly correlated with HCC development by univariate analysis (30).

The level of α-FP 200 ng/ml was considered diagnostic for HCC and the increase in serum levels of α-FP up to 200 ng/ml in a cirrhotic patient at presentation predicts the development of HCC in these patients (31).

High serum TGF-β level is common in cirrhotic patients with HCC than in non-HCC and that means TGF-β level is related to the severity of the disease. High serum TGF-β level is a significant predictor of bad outcome in patients with HCC. Therefore, measurement of serum TGF-β level provide a new strategy to more aggressive treatments for high-risk groups.

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Conflict of interests

All authors declare no conflicts of interest.

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