Clinical value of transforming growth factor beta as a marker of Fibrosis in adolescents with Chronic Liver Diseases

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Abstract: Background: Hepatic fibrosis is the final common path of liver injury in most chronic liver diseases and can lead to cirrhosis, which is responsible for the majority of clinical complications. Our aim is to asses the clinical value of serum Transforming growth factor β (TGFβ) as a fibrogenesis marker in adolescents with chronic Liver Diseases**. Methods:** We measured serum levels of TGF-β in 25 adolescents with chronic liver disease and 25 healthy controls, and determined their relationship to frequently used liver function tests and liver biopsy findings. **Results:** Serum Transforming growth factor β was significantly higher in patients than in controls as ($P < 0.001$). Significant positive correlation between TGFβ and TSB as r is 0.4682 and p is < 0.05. High significant positive correlation between TGFβ and (stage, grade of liver fibrosis, PT and duration of illness)as p is < 0.001and r is 0.9409, 0.7447, 0.5293 and 0.5952 respectively. Highly significant negative correlation with prothrombin concentration (PC) and serum albumin level as p is < 0.01 and r is -0.6460 and -0.5371 respectively. Sensitivity of TGFβ in diagnosis of fibrosis was 65% , specificity 94% and area under curve (AUC) was 0.812.The cut-off value of TGFβ used to discriminate significant fibrosis was 22.6 ng/ml and it was a dependant predictor factor for diagnosis of fibrosis with positive predictive value 75.5% and negative predictive value 90.4 %. **Conclusions:** TGF-β had the ability to discriminate patients with significant fibrosis. and may be useful in reducing but not replacing the need for liver biopsy.

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Introduction:

Chronic liver diseases (CLDs) are defined as the continuity of clinical and biochemical evidence of hepatic dysfunction for longer than six months ***(1).***.Hepatitis B and C (HBV, HCV) are, and will remain for some time, major health problems in Egypt. Both infections can lead to an acute or silent course of liver disease **(2).** Hepatic fibrosis is the final common path of liver injury in most chronic liver diseases and can lead to cirrhosis, which is responsible for the majority of clinical complications. Fibrosis is characterized by excess deposition of extra-cellular matrix (ECM) components including different collagens and non-collagenous proteins such as laminin, fibronectin, undulin, and so on (3). The key cellular mediator of fibrosis is the hepatic stellate cells (HSCs) which when activated serve as the primary collagen-producing cell. Hepatic stellate cells (HSCs) are activated by a variety of mechanisms, including cytokines, chemokines and others (**4**). Transforming growth factor-beta (TGF-β) is released by activated HSCs and is considered the key profibrogenic cytokine in liver disease, with participation in most critical events during liver fibrogenesis **(5).** Liver biopsy is essential in establishing the diagnosis, and in assessing the degree of fibrosis. Although liver biopsy has long been considered to be the gold standard of fibrosis assessment, the procedure is invasive, potentially associated with complications, and provides only a semi-quantitative assessment. Its repetition is required for following up the disease progression and monitoring treatment efficacy. This repetition is not easily accepted by patients especially in the pediatric population. Moreover, the diagnostic accuracy of liver biopsy in the staging of fibrosis is seriously affected by errors in sampling and inter-observer variation (**6) and (7**). These drawbacks justify an intensive search for non-invasive alternatives that are safe, inexpensive and reliable (**8**). Non-invasive diagnosis of liver fibrosis has been extensively evaluated in adult populations (**9), (10) & (11)**. In contrast, in pediatric population, data are lacking and liver biopsy is still the only reliable tool for diagnosing the histological features (12). **Our aim is** to asses the clinical value of serum Transforming growth factor β (TGFβ) as a fibrogenesis marker in adolescents with chronic Liver disease.

Subjects and Methods:

A mixed retrospective and prospective study was conducted from October 2009 to June 2010.Twenty five patients with chronic liver disease (group 1) were chosen from hepatology clinic of Benha university hospital (Kalyobia Governate)and the Liver Institute (Menofia Governate). Twenty five healthy children matched for age, sex, locality and socioeconomic state served as control (group 2).

Written consent was taken from parents before including their children in the study**.** Patients were included if their ages ranged from 10-16 years and with chronic liver disease. Patients with gastrointestinal bleeding (acute attack), chronic renal failure, hepatic encephalopathy were excluded from the study. All cases were subjected to: full history taking, complete clinical examination including ; Liver (surface, edge, consistency and span), spleen(surface, edge, consistency and size), presence or absence of ascites. Presence or absence of manifestation of liver cell failure (edema, bleeding tendency, jaundice and angiomas).Laboratory investigations including: complete blood count,fasting blood sugar, blood urea and creatinine, liver function tests including(ALT, AST, serum bilirubin (total and direct), serum albumin, prothrombin time and concentration. TGFβ was measured using DRG TGFβ ELISA kit. Ultrasonography-guided liver biopsy was done for chronic hepatitis patients. Liver biopsies were performed using true cut needle. Biopsy specimens were fixed in formalin and embedded in paraffin. Liver fibrosis and necroinflammatory activity were evaluated according to Ishak staging and grading score where histological activity index (HAI) ranged from 0 to 12, while fibrosis score ranged from F0 to F6 (**13).**

Results:

Demographic data among studied groups, including sex, residence and mean age (13.06±2.5) years in group 1 compared to (13.1±2.1) years in group 2 are shown in table (1).Chronic hepatitis C was the most common etiology of chronic liver disease among our cases (44%) followed by autoimmune hepatitis (24%),chronic hepatitis B (16%),glycogen storage disease (8%),congenital hepatic fibrosis and Alpha1 anti-trypsin deficiency (4%) each as shown in table (2). Table (3) shows clinical characteristics of studied cases. Table (4) shows laboratory data of studied groups and revealed highly significant difference between patients group and control group as regard serum transaminases level, albumin level ,total &direct billirubin, prothrombin time and concentration, also serum level of TGFβ was highly significantly elevated in group (1)than in group (2) as ($P < 0.001$). Grading of liver fibrosis revealed that; there were 8,9,5,2 and 1 patients in grade 2, 3, 4, 7and 8 respectively. Staging of liver fibrosis revealed that; there were 8, 7, 2, 5 and 3 patients in stage **1**, **2** , **3**, **4** and **5** respectively. Figure (1) shows significant positive correlation between TGFβ and TSB as r is 0.4682 and p is < 0.05. High significant positive correlation between TGFβ and (stage, grade of liver fibrosis ,PT and duration of illness)as p is < 0.01 and r is 0.9409 , 0.7447, 0.5293 and 0.5952 respectively are shown in figures 2,3,4and 5. Highly significant negative correlation with PC and serum albumin level as p is < 0.01 and r is -0.6460 and -0.5371 respectively as shown in figures 6 and 7. Sensitivity of TGFβ was 65% , specificity 94% and area under curve (AUC) was 0.812 as shown in figure 8.The cut-off value of TGFβ used to discriminate significant fibrosis was 22.6 ng/ml and it was a dependant predictor factor for diagnosis of fibrosis with positive predictive value 75.5% and negative predictive value 90.4 %.

Table (1) Demographic data among studied groups

	Cases(25)	**Control(25)**	**Z / (t)**	**P**
Age (years)				
Range	10-16	10-16		
Mean ±S.D	13.06±2.5	13.1±2.1	0.45	> 0.05
Sex				
Male	13 (52%)	11 (44%)	0.56	
female	12 (48%)	14 (56%)		> 0.05
Locality				
Urban				
No	10	11		
%	40	44		
rural			0.28	
No	15	14		> 0.05
%	60	56		

Table (2) Distribution of studied cases regarding the etiology of liver disease

Etiology	Frequency	
	No	%
Chronic Hepatitis C	11	44
AIH (Autoimmune hepatitis)	6	24
Chronic Hepatitis B	4	16
GSD1(Glycogen storage disease type 1)	2	8
Congenital hepatic fibrosis	1	4
Alpha1 anti-trypsin deficiency	1	4
Total	25	100

Table (3) Clinical characteristics of studied cases

	Frequency	
	No	%
Hepatomegaly	16	64
Splenomegaly	16	64
Jaundice	10	40
Pallor	7	28
Portal hypertension	5	20
Ascites	3	12
Lower limb edema	1	4

Table (4): Laboratory data in studied groups

	Group 1 (25)	Group 2 (25)	(t)	P
AST (IU/L)				
§ **Range**	12- 430	17- 40		
§ **Mean+S.D.**	72.3+ 88	23.3+ 5.6	2.7	< 0.01**
ALT (IU/L)				
§ **Range**	10- 625	15- 36		
§ **Mean+ SD**	77.4± 120	22.8± 6.1	2.25	< 0.05*
Total billirubin mg/dl				
§ **Range**	0.3- 6.6	0.2- 1		
§ **Mean+SD**	2.6±1.8	0.66±0.24	5.4	< 0.001**
Direct billiruban (mg/dl)				
§ **Range**	0.09-2.1	0.01-0.18		
§ **Mean+SD**	0.81 + 0.71	0.056+0.05	5.3	< 0.001**
S albumin (gm/dl)				
§ **Range**	2.1-4.8	3.8 – 5.1		
§ **Mean+SD**	3.3 + 0.72	4.43 + 0.44	6.2	< 0.001**
Prothrombin time PT(sec)				
§ **Range**	11.5- 18	11- 13		
§ **Mean+SD**	13.4±1.8	12.2±0.44	3.2	< 0.01**
Prothrombin concentration PC %				
§ **Range**	54- 100	95- 110		
§ **Mean±SD**	84.8±16.9	99±3.1	4.2	< 0.01* *
TGFβ (ng/ml)				
Range	12.3- 45.1	9.8- 20.7		
Mean±SD	25±10.3	14.9±3	4.6	< 0.001**

* significant value; ** high significant value

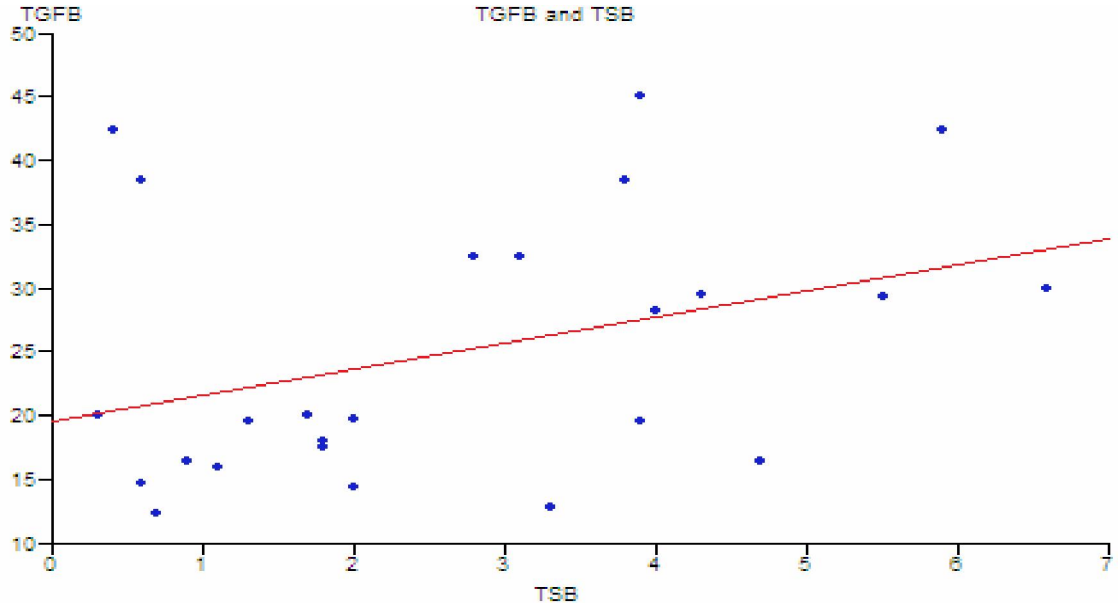

Figure 1: Correlation between TGFβ and TSB.

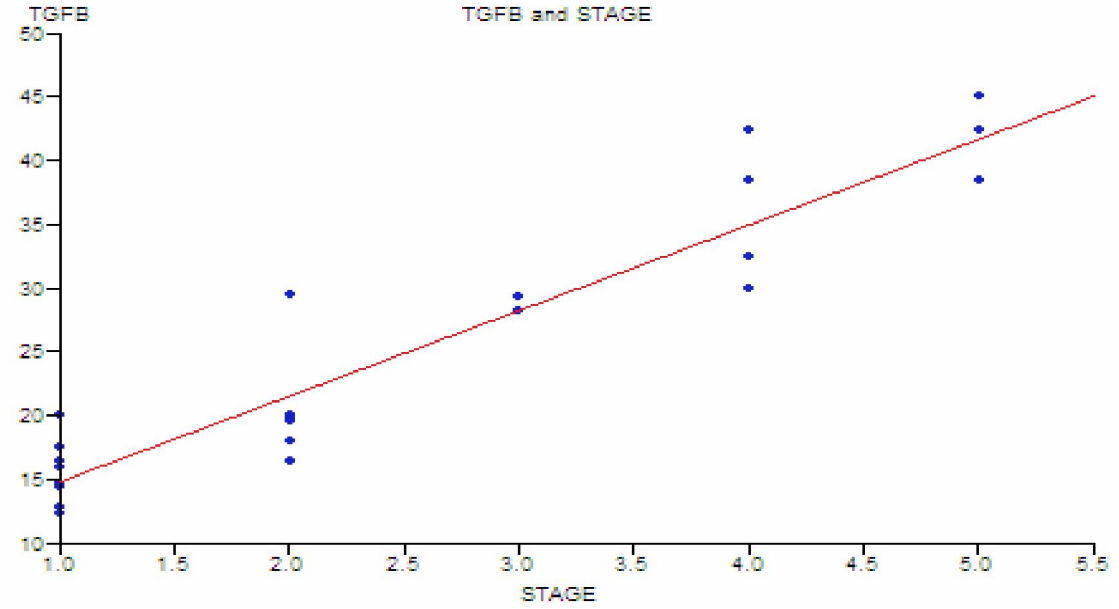

Figure 2: Correlation between TGFβ and stage of liver fibrosis

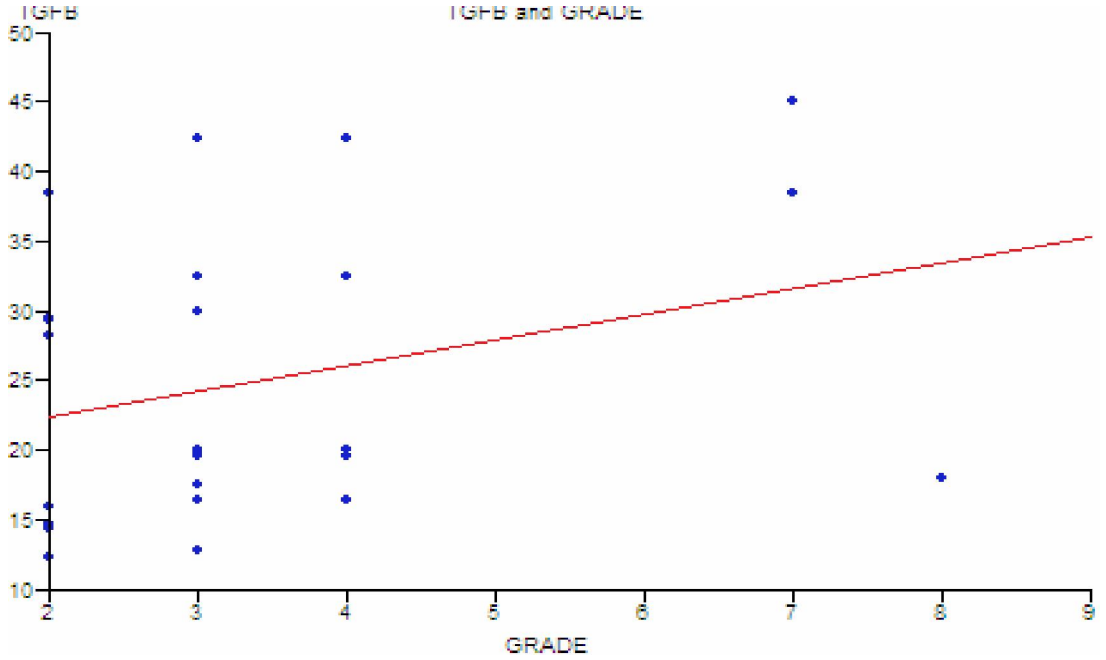

Figure 3: Correlation between TGFβ and grade of liver fibrosis

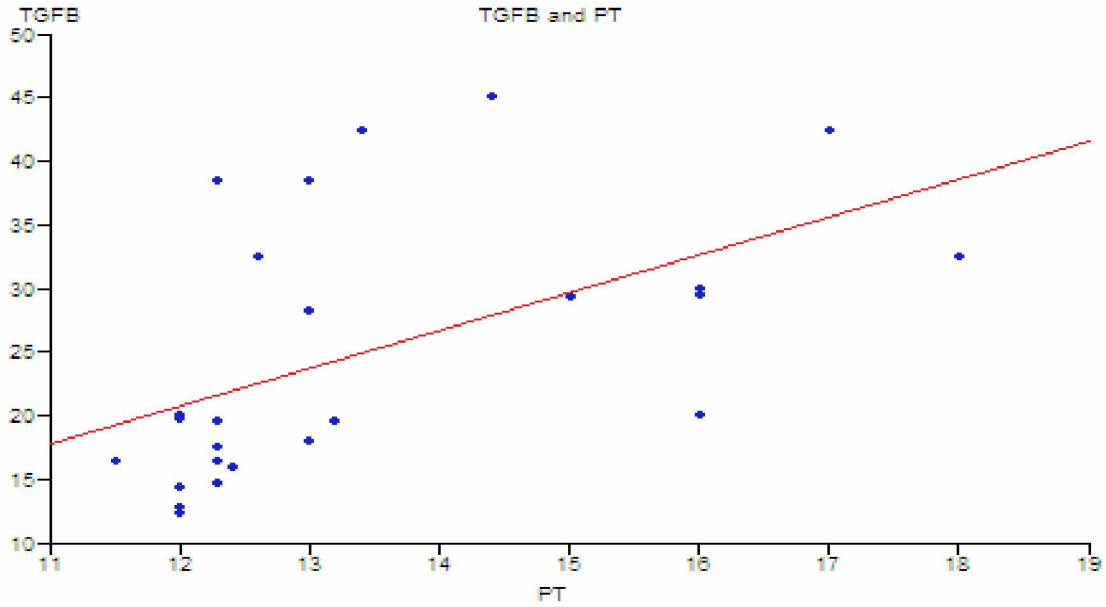

Figure 4: Correlation between TGFβ and PT.

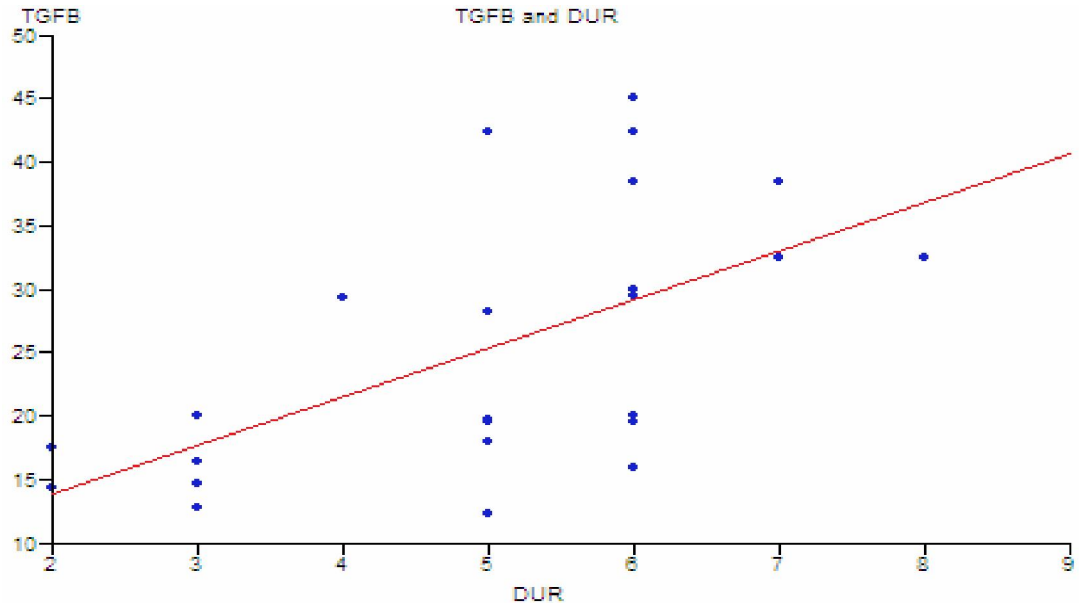

Figure 5: Correlation between TGFβ and duration of illness.

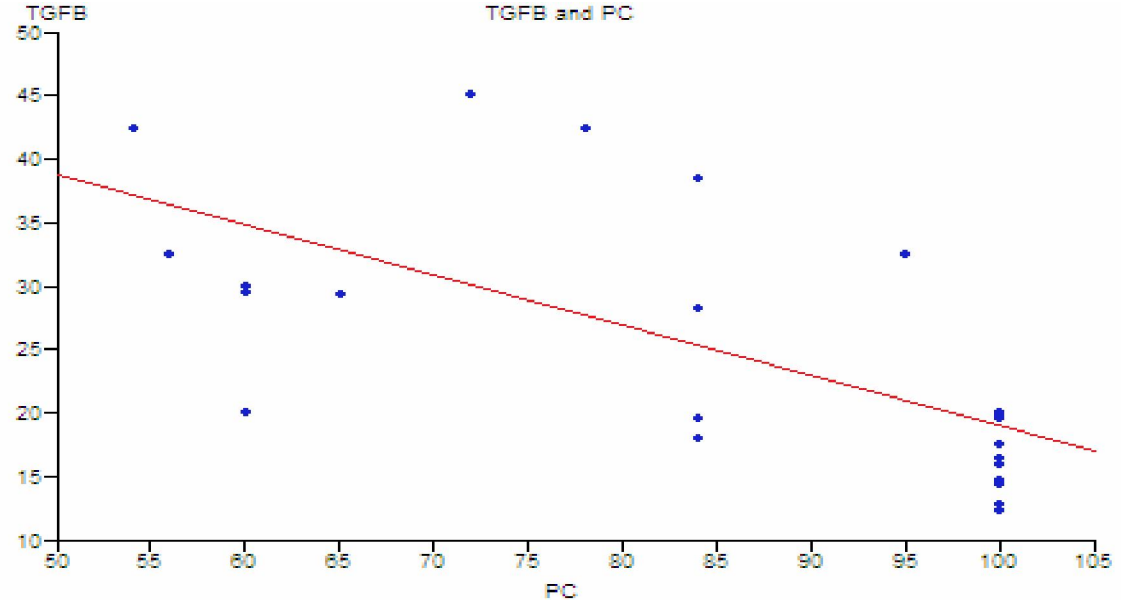

Figure 6: Correlation between TGFβ and PC.

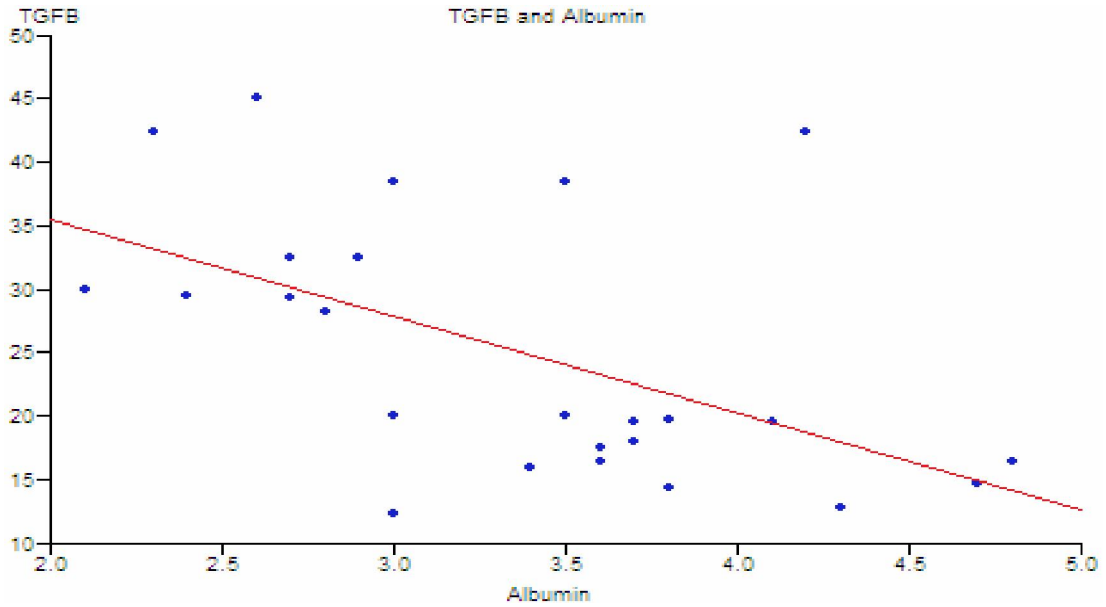

Figure 7: Correlation between TGFβ and serum albumin level.

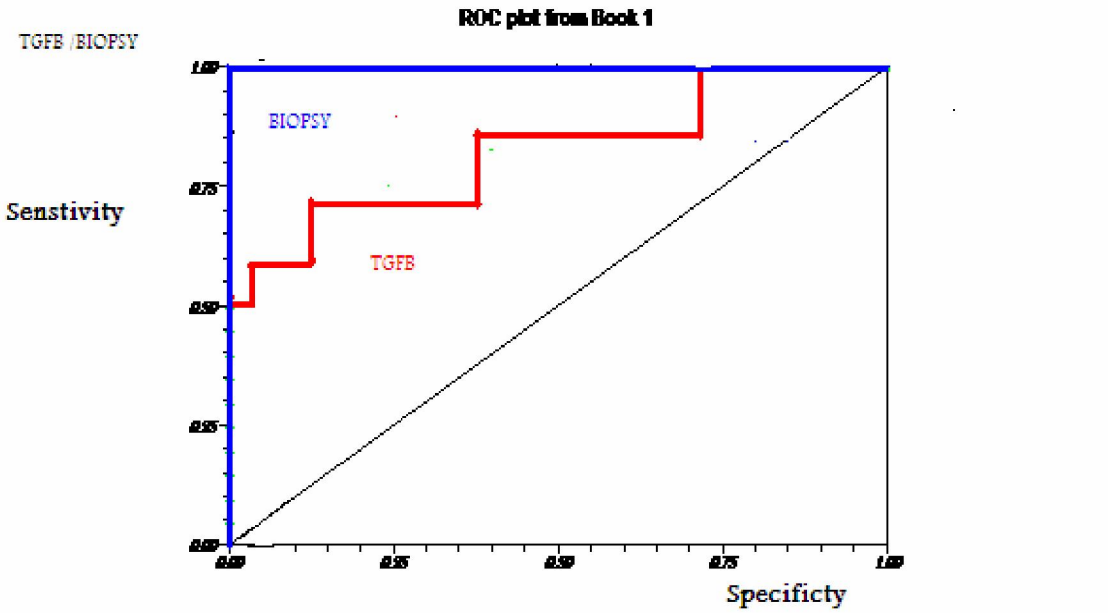

Figure 8:Nonparametric receiver operating charachteristic(ROC) curve for assessing the diagnostic value of TGFβ as an indicator of liver fibrosis.

Discussion

In the normal liver, HSCs express very little TGF-β, and hepatocytes essentially none. When injury strikes, inflammatory cells are drawn to the site of injury and HSCs undergo activation and becoming fibrogenic **(14)**,(**15**).

The current study revealed that, serum level of TGF-β was highly significantly elevated in patients with chronic liver disease than control. This significant elevation might reflect the fibrogenic process in the liver.

Luo et al.,2001(16) found a significant elevation of TGF-β1 in liver cirrhosis, yet its correlation with activity was moderate.In another Egyptian study done by **Abdel-Ghaffar et al.,2010 (17)** they found that; TGF-β1 was significantly increased in children with chronic liver disease than control. There are conflicting results in literature as to which TGF-beta level increase or remain unchanged in patients with chronic hepatitis.

Hong-Lei Weng et al., 2009(18), reported elevated TGF-β1 serum levels in patients with chronic hepatitis B virus (HBV)/hepatitis C virus (HCV) infections. On the other side **Liberek et al.,2009(19)** reported that ; in chronic hepatitis group of patients the plasma TGF-beta level did not differ from the control group and did not correlate with grading and staging of the liver tissue fibrosis. These contradictory results may be attributed to the heterogeneity of populations, races, diseases, and other confounding factors. Our results showed that,TGF-β correlated positively with PT, TSB, stage and grade of liver fibrosis and negatively with PC and serum albumin levels. Our results are in agreement with **Flisiak and Prokopowicz**, **2000(20)** as they found a correlation between elevated TGF-β and impairment of some synthetic liver functions, and ***Filiask et al., (2002)***(21)who reported that, TGFβ correlated significantly positively with liver fibrosis. Also in accordance with our results, **Iagoda et al. , 2006(22)** studied correlations between growth factors and histological changes in the liver in 48 patients with chronic viral hepatitis and hepatic cirrhosis ,they showed that the blood level of transforming growth factor-beta1 (TGF-beta1) increases according to increase in histological activity and the degree of hepatic fibrosis and that there is a positive correlation between TGF-beta1 and the degree of hepatic inflammation and fibrosis. The stimulatory effect of TGF-beta on collagen synthesis by fat-storing cells is observed *in vitro* at a concentration of 10 ng/ml **(20)**. In our study, the level of circulating TGF-beta was two fold increase and a level more than 22.6 ng/ml had a sensitivity of 65% and specificity of 94% in identifying significant fibrosis. In the study of **Abdel-Ghaffar et al.,2010(17)** they found that, TGF-β1 more than 54.8 ng/ml had a sensitivity of 78.6% and specificity of 71.4% in identifying significant fibrosis. The difference in the results between our study and other studies could be explained by the difference in the mean age of the cases and accordingly the aetiology of chronic liver disease. **Hong-Lei Weng ,2009**(18) reported that,TGF-β1/Smad2 signaling in liver fibrogenesis is not a generalized feature and detected in an etiology-dependent manner.

In conclusion, TGF-β may be used to predict significant fibrosis and/or cirrhosis in children with chronic hepatitis B & C and other causes of chronic liver disease. That is to say, non-invasive markers will likely reduce but not replace the need for liver biopsy, which may be useful in monitoring of disease development and treatment effectiveness and might be an inseparable part of assessment of chronic hepatopathies.

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