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Research Article

Assessment of Connective Tissue Growth Factor in the Diagnosis of Hepatic Fibrosis in Chronic Hepatitis B: A Correlation Analysis

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Abstract

Hepatitis B Virus (HBV) infection is a serious global health problem. About 350 million people throughout the world are chronically infected with hepatitis B virus (HBV) infection. HBV is responsible for 76.3% of cases of chronic hepatitis and 61.15% of cases of cirrhosis in Bangladesh. This cross sectional study was carried out in the Department of Clinical pathology, in collaboration with Department of Hepatology and Department of pathology, BSMMU from July'2015to June 2016. Clinically suspected case of chronic hepatitis B and adult age group was included in the study. In this study we explored the correlation between serum CTGF and stages of hepatic fibrosis. Histopathology was considered as gold slandered. Serum CTGF was tested by Enzyme Linked Immunosorbent Assay (ELISA). Other markers such as Transforming Growth Factor beta (TGFβ1), Hyalunoricacid (HA) were tested. Spearman's rank correlation coefficient test was used for correlation analysis. All statistical computations was performed by using SPSS 17.0. The mean age was found 29.92 ± 6.17 years which was not statistically significant (p>0.05) in four groups as like gender. Significant linear positive correlation was found between serum CTGF, TGF-β1 and HA level and stages hepatic fibrosis. Result shows serum CTGF level cut off value of (≥56.6 ng/ml) as the value with a best combination, the area under the ROC curve for CTGF 0.875 for identification of hepatic fibrosis. CTGF can be used as more reliable diagnostic tool than TGF-β1 and HA for the assessment of hepatic fibrosis in patients with CHB.

Keywords: Connective tissue growth factor; Liver fibrosis; Chronic hepatitis B; ELISA

Introduction

Chronic Hepatitis B (CHB) may be defined as chronic necroinflammatory disease of the liver caused by persistent infection with hepatitis B virus. About 500,000 to 1.2 million people die annually from liver disease. It is the 10th leading cause of death in world [1]. HBV is responsible for 76.3% of cases of chronic hepatitis and 61.15% of cases of cirrhosis in Bangladesh. It poses huge burden on the health of our patients [2]. Transforming Growth Factor Beta (TGFβ1) is a major cytokine associated with activation of hepatic stellate cell and extracellular matrix deposition. There is a close relation between TGFβ and Connective Tissue Growth Factor (CTGF). Transforming growth factor β (TGF1 β) and connective tissue growth factor are involved in fibrogenesis. CTGF as a downstream effector of TGFβ1-induced extracellular matrix production and fibroblast proliferation [3]. Liver biopsy is the gold standard to evaluate the histological stages of hepatic fibrosis and an integral part of management of chronic hepatitis B; but the procedure is invasive, blind and costly. It carries definite risk of occasional complications such as pain, hypotension, intraperitoneal bleeding, injury to the biliary system and even death. In liver biopsy sampling variation may occur. Histological evaluation is dependent on experienced histopathologist [4]. Additionally, fibrosis is not equally distributed in the liver of some patients with liver disease. Fibrosis is missed on a single liver biopsy in 10%-30% of cases [5]. CTGF is simple, quick, less expensive method can be carried out in peripheral hospital where less chance of sampling error. Several non-invasive markers have been reported to predict the presence of significant fibrosis in patients with chronic hepatitis B. But most of these markers require complicated calculations, making them less accessible to clinicians. Among this AST/ALT ratio, AST to Platelet Index Ratio (APRI) and Age Platelet Index ratio (API) are based on routine laboratory results. Recently several clinical studies have been attempted to identify serum markers like Connective Tissue Growth Factor (CTGF), Transforming Growth Factor Beta (TGFβ1), hyalunoric acid, laminine, procollagen III, collagen IV, matrix metalloproteinases, and imaging technique like fibroscan that correlate with the degree of fibrosis. These non invasive markers could be used in conjunction with liver biopsy [6]. Among these non invasive markers, CTGF shows more diagnostic sensitivity and specificity [3,7]. Fibrogenesis is difficult to heal when it reaches the middle or later stages. Progression of fibrosis can be delayed by specific antiviral therapy. Therefore a simple, sensitive and non invasive method is needed to assess the prognosis and stages of hepatic fibrosis. It reduces the need for repeated liver biopsies. Serum CTGF levels correlated with the stages of hepatic fibrosis and become an important non invasive marker of hepatic fibrosis other than serum Transforming Growth Factor Beta (TGFβ1), Hyalunoric Acid (HA) [8]. Therefore, in this study measurement of serum CTGF was

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done as a noninvasive marker in the diagnosis of hepatic fibrosis in Chronic Hepatitis B.

Materials and Methods

This cross sectional study was conducted at the Department of Laboratory medicine in collaboration with Department of Hepatology and Department of Pathology, Bangabandhu Sheikh Mujib Medical University, Dhaka from July 2015 to June 2016. Forty patients who fulfilled the inclusion criteria of CHB attended in the Department of Hepatology, BSMMU, were conveniently included in this study. Patients having any condition like decompensated cirrhosis of liver, co-infected with hepatitis C virus infection, antiviral therapy, nonalcoholic fatty liver disease and hepatocellular carcinoma were excluded from the study. After taking informed consent, a careful history and the details information were recorded by the investigator in a preformed data sheet. With all aseptic precaution, 5 ml venous blood was taken before liver biopsy, allow to clot and separate serum by centrifugation at room temperature. The serum was stored at -20°c until analysis. Serum CTGF, TGF Beta-1 and HA were measured in the Department of Clinical Pathology by using Enzyme Linked Immuno Sorbent Assay (ELISA) based on sandwich principle. Cut-off value of serum CTGF ≤ 56.6 ng/mL (DRG CTGF ELISA EIA-5195, 2011). Cut-off value of serum TGF Beta-1 ≤600 pg/mL (DRG TGF Beta-1 ELISA EIA-1864, cut-off value of serum hyalunoric acid ≤ 75ng/mL (DRG CTGF ELISA EIA-5195, 2009)

Needle liver biopsy was done in the Department of Hepatology by Hepatologist through right 8th or 9th intercostals space with 14Fr, 15cm Tru-cut biopsy needle. Biopsy material was fixed in 10% formalin. The specimen was sent to the Department of Pathology, BSMMU for complete histopathological examination. Haematoxyline & Eosin and Masson's trichrome stains were done to see the different stages of hepatic fibrosis by Metavir scoring system.

Stages of fibrosis

The fibrosis score is also assigned a number from 0-4

O= no fibrosis

F1 =portal fibrosis without septa

F2=portal fibrosis with a few septa

F3=numerous septa without cirrhosis

F4= cirrhosis

Results

This cross sectional study was carried out in the Department of Laboratory medicine, BSMMU, Dhaka. The cases were included in the study from the Department of Hepatology, in BSMMU. We investigated 40 Chronic Hepatitis B (CHB) patients without having any condition like decompensated cirrhosis of liver, co-infected with hepatitis C virus infection, antiviral drug therapy, nonalcoholic fatty liver disease and hepatocellular carcinoma. Histopathology was gold standard to identify the stages of hepatic fibrosis. According to fibrosis the patients were grouped into four, F0=13, F1 =6, F2=17, F3=4 patients were found in each group (Table 1).

A total of 40 patients with chronic hepatitis B were included in this study. Maximum patient's age were belonged to 20-29 years in four groups. The mean age was found 29.92 ± 6.17 years with range from 19 to 52 years in F0 group, 29.00 ± 7.35 years with range from 18 to 52 years in F1 group, 27.53 ± 5.79 years with range from 19 to 50 years in F2 group and 30.50 ± 14.48 years with range from 18 to 50 years in F3 group. The mean age difference was not statistically significant (p>0.05) in four groups (Table 2).

Table 1: Distribution of the study population by age among hepatic fibrosis group (n=40).

Age (in year)	Stages of Fibrosis								p value*
	F0		F1		F2		F3		
	No.	%	No.	%	No.	%	No.	%	
≤20	0	.0	0	.0	3	17.6	2	50	
20-29	7	53.8	4	66.7	8	47.1	0	0	
30-39	4	30.8	2	33.3	6	35.3	1	25	
≥40	2	15.4	0	.0	0	0	1	25	
Mean ± SD	29.92 ± 6.17		29.00 ± 7.35		27.53 ± 5.79		30.50 ± 14.48		0.787ns

*ANOVA test was done to measure the level of significance
ns = not significant

Table 2: Distribution of the study population by sex among hepatic fibrosis group (n=40).

Sex	Stages of Fibrosis								p value*
	F0		F1		F2		F3		
	No.	%	No.	%	No.	%	No.	%	
Male	9	69.2	5	83.3	15	88.2	3	75.0	0.622ns
Female	4	30.8	1	16.7	2	11.8	1	25.0	

*Chi square test was done to measure the level of significance.
ns = not significant

Table 3: Correlations of CTGF, TGF-β1 and HA with stages of fibrosis.

Stages of Fibrosis *vs*	r value	p value*
CTGF	0.708	0.001s
TGF-β1	0.555	0.001s
HA	0.643	0.001s

*Spearman's rank correlation was done to measure the level of significance.
s = significant

Regarding the gender distribution of the study patients, male were predominant in four groups, which was 9 (69.2%) in F0 group, 5 (83.3%) in F1 group, 15 in F2group (88.2%) and 3 (75.0 %) in F3 group. The difference was not statistically significant (p>0.05) between four groups (Table 3).

Enzymes Linked Immunosorbent Assay (ELISA) measured serum CTGF, TGF-β1 and HA level of 40 patients with chronic hepatitis B were expressed in ng/mL for CTGF, pg/mL for TGF-β1and ng/mL for HA. Stages of hepatic fibrosis evaluated by histopathology which was expressed in category. Significant correlation was found between serum CTGF, TGF-β1 and HA level and hepatic fibrosis. The value of Spearman's rank correlation coefficient was 0.708, 0.555 and 0.643 respectively which is positive significant correlation (p<0.001). Therefore, there was linear positive correlation between serum CTGF, TGF-β1 and HA level and stages hepatic fibrosis (Figure 1).

The area under the Receiver-Operator Characteristic (ROC) curves for the hepatic fibrosis predictors are depicted in (Table 1). Receiver-Operator Characteristic (ROC) were constructed using

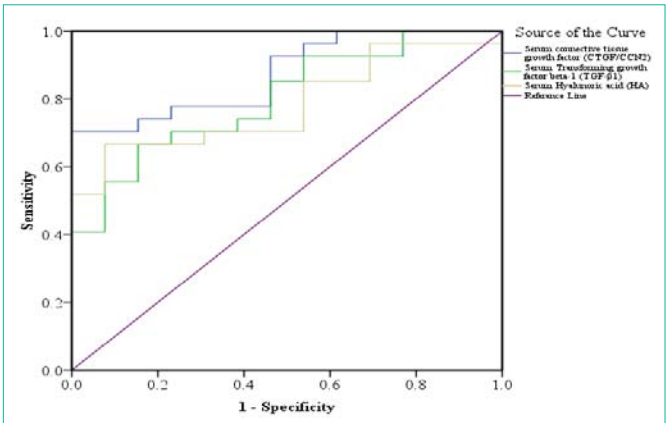

Figure 1: ROC Curve of CTGF, TGF-β1 and HA by Fibrosis.

serum CTGF, TGF-β1 and HA level of the patients with chronic hepatitis B. It shows serum CTGF level cut off value of (≥56.6 ng/ml) as the value with a best combination, the area under the ROC curve for CTGF 0.875 for identification of hepatic fibrosis.

Discussion

This cross sectional study was carried in Department of Clinical Pathology. We measured the concentration of serum CTGF in patients with Chronic Hepatitis B (CHB) and investigated the correlation between serum CTGF concentration and stages of hepatic fibrosis. In this study, we investigated 40 CHB patients without having any condition like decompensated cirrhosis of liver, co-infected with hepatitis C virus infection, antiviral drug therapy, nonalcoholic fatty liver disease and hepatocellular carcinoma. 40 CHB patients have serum HBsAg positive for more than 6 months were enrolled for the study. There are substantial number of publication correlating the serum CTGF and stages of hepatic fibrosis in the world [7,9,10]. But there is no known study done in correlation of serum CTGF and stages of hepatic fibrosis in Bangladesh. It is our little endeavor to correlate the serum CTGF concentration and stages of hepatic fibrosis in chronic hepatitis B. In this study mean age was found 29.92±6.17 years in Fo group, 27.14±7.65 years in F1 group and 28.45±9.05 years in F3 group. In this study, the highest incidence of CHB patients was found at 20-29 age groups. Alam et al., (2008) found that chronic hepatitis B affects the younger population (age group 21-30) of Bangladesh. This finding was similar with our study [11]. Analysis of gender distribution showed out of 40 patients 32 were male and 8 patients were female. Male female ratio 4:1. Male were found 9(69.2%) in F0 group, 5(83.3%) in F1 group 15(88.2%) in F2 group and 3(75%) F3group. In this current study, male were predominant in three groups. Rahman (2011) observed that males were predominant in chronic hepatitis B patients in Bangladesh which was consistent with our study [12]. A Spearman's rank correlation was performed to determine the correlation between serum CTGF and stages of hepatic fibrosis in this study. Spearman's rank correlation analysis showed that correlation coefficient was 0.652, which is positive significant correlation ($p<0.001$).This correlation suggested that CTGF is related to severity of liver fibrosis. Similar finding was observed from the study of Die et al. They found the correlations coefficients between serum CTGF and fibrosis stage in patients with Chronic hepatitis B was 0.681($p<0.001$). So, our result was in accordance with above published study [3]. In this study we detected correlation between TGF-β1 and hepatic fibrosis. Spearman's rank correlation coefficient test was done. Positive Spearman's correlation was found between serum TGFβ1 and hepatic fibrosis. The value of correlation coefficient was 0.5550, which is positive correlation and statistically significant ($p<0.001$). This finding is similar to the study done by Peter et al., (2002) [13]. They found significant correlation between TGF-1 mRNA expression and the histological grade of cystic hepatic fibrosis ($r=0.78$, $p<0.005$, demonstrating a definitive role for TGF- β1 in the pathogenesis associated with CFLD. In this current study we also detected correlation between HA and hepatic fibrosis. Positive Spearman's correlation was found between serum HA and hepatic fibrosis. The value of correlation coefficient was 0.643, which is positive correlation and statistically significant ($p<0.001$). This finding is similar to the study done by Shahria et al., (2016) [14]. They found positive correlation between HA and hepatic fibrosis ($r=0.248$, $p=0.013$). They quantified Hepatic fibrosis by morphometry. The area under the Receiver-Operating Characteristic (ROC) curves for the hepatic fibrosis was depicted in our study. The Area Under the ROC Curve (AUC) for CTGF, TGF-β1 and HA were 0.875, 0.801, 0.783 respectively in current study. Qiu et al., (2010), Nawar et al., (2011) and Avila et al., (2009) showed AUC for CTGF was 0.681; TGF-β1 was 0.812 and HA was 0.808 respectively. So, our observation in this study was within international norms [7,15,16].

Conclusion

Our study revealed that CTGF, TGF-β1 and HA are simple, quick, less expensive and non invasive direct biomarkers. In this study, we found that correlation coefficient and AUC of CTGF is higher than TGF-β1 and HA. We concluded that serum CTGF can be used as more reliable diagnostic tool than TGF-β1 and HA for the assessment of hepatic fibrosis in patients with CHB. CTGF can be used for early detection, reduce progression, assess the prognosis in different stages of hepatic fibrosis. It can also be used as alternative of liver biopsy for the diagnosis of hepatic fibrosis in CHB where biopsy and histopathological examination are not possible.

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