Role of Alanine Transaminase, Prothrombin time and HBV-DNA in the Diagnosis of Chronic Hepatitis B Induced Hepatic Fibrosis

*Ferdoushi S,[1] Mortaz R,[2] Islam MS,[3] Paul D,[4] Parvin S,[5] Sultana T,[6] Rahman MQ,[7] Nurullah A,[8] Talukder SI,[9] Sarker S,[10] Ahmed AN[11]

Non-invasive markers play an important role in the diagnosis of hepatic fibrosis in chronic hepatitis B (CHB). The aim of this study was to determine the correlation of serum Alanine transaminase (ALT), Prothrombin time (PT) and Hepatitis B virus DNA load (HBV-DNA) with stages of hepatic fibrosis and explored the clinical value of ALT, PT and HBV-DNA in the assessment of hepatic fibrosis in CHB. This cross sectional study was done in department of Clinical Pathology, Bangabandhu Sheikh Mujib Medical University, Dhaka from March, 2012 to February, 2013. 40 CHB patients admitted in the Department of Hepatology, BSMMU were included in this study. Serum ALT, PT and HBV-DNA reports were collected from patients' files. Biopsy material was sent to the Department of Pathology, BSMMU for complete histopathological examination. The sensitivity of ALT, PT and HBV-DNA were 56.3%, 6.06, 37.5 % and specificity 50.0%, 85.7, 75.0%, respectively. Spearman's rank correlation coefficient were 0.164, -0.087 and -0.129 between serum ALT, PT and HBV-DNA with stages of hepatic fibrosis (P>0.05). Present data revealed that serum ALT, PT and HBV-DNA in CHB were not associated with stages of hepatic fibrosis. So we concluded that serum ALT, PT and HBV-DNA are not useful diagnostic tool for assessing the hepatic fibrosis in CHB.

[Dinajpur Med Col J 2015 Jul; 8 (2):204-211]

Key words: Alanine transaminase, Prothrombin time, HBV-DNA, Chronic hepatitis B, Hepatitis B virus, Hepatic fibrosis

Introduction

Hepatitis B virus (HBV) infection is a serious global health problem. About 400 million people throughout the world are chronically infected with hepatitis B virus infection. Chronic Hepatitis B (CHB) may be defined as chronic necroinflammatory disease of the liver caused by persistent infection with hepatitis B virus.[1] It causes a spectrum of different disease ranging from clinically asymptomatic carrier state to the

1. *Dr. Sheuly Ferdoushi, Assistant Professor, Department of Gastroenterology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh.*
2. *Dr. Rubaiyat-E-Mortaz, Research Assistant, Department of Clinical Pathology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh*
3. *Dr. Md. Saiful Islam, Assistant Professor, Department of Clinical Pathology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh.*
4. *Dr. Debatosh Paul, Assistant Professor, Department of Clinical Pathology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh*
5. *Dr. Silpi Pervin Assistant professor Department of Pathology, MH Samorita Medical College, Dhaka*
6. *Dr. Tuhin Sultana, Associate Professor, Department of Clinical Pathology, BSMMU Dhaka, Bangladesh*
7. *Professor Md. Quddusur Rahman, Professor, Department of Clinical Pathology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh*
8. *Dr. AFM Nurullah, Assistant Professor, Department of Radiology & Imaging, Dinajpur Medical College, Dinajpur*
9. *Dr. Sadequel Islam Talukder, Assistant Professor, Department of Pathology, Dinajpur Medical College, Dinajpur*
10. *Dr. Shibesh Sarker, Associate Professor, Department of Radiology & Imaging, Dinajpur Medical College, Dinajpur*
11. *Professor AN Nashimuddin Ahmed, Professor and Chairman, Department of Clinical Pathology, BSMMU, Dhaka, Bangladesh.*

** For correspondence*

development of cirrhosis and hepatocellular carcinoma.[2] Patients with significant hepatic inflammation and fibrosis are at the highest risk of developing complication. In Bangladesh prevalence of HBV infection in healthy population is 5.4%.[3] The lifetime risk of HBV infection in these areas is 20%-60%. HBV is responsible for 76.3% of cases of chronic hepatitis and 61.15% of cases of cirrhosis in Bangladesh.[4]

Liver fibrosis is primary complication of chronic hepatitis.[5] Hepatic stellate cells are the key cellular source of extracellular matrix in liver. Following liver injury of any etiology, stellate cells undergo activation. The activated stellate cells undergo a series of phenotypic changes that collectively lead to the accumulation of ECM resulting in hepatic fibrosis.[6]

Liver biopsy is the gold standard to evaluate the histological stages of hepatic fibrosis and an integral part of management of CHB; but the procedure is invasive, blind and costly. It carries definite risk of occasional complication. In liver biopsy sampling variation may occur as it is not equally distributed in the liver and missed on a single liver biopsy in 10%-30% of cases.[7] Histological evaluation is also dependent on experienced histopathologist.[8]

Development of serum markers offers an attractive, cost effective alternative to liver biopsy for both patients and physicians. They are commonly divided into direct and indirect markers. Direct markers are fragments of the liver matrix components produced by hepatic stellate cells (HSC) during the process of ECM remodeling. Indirect markers include molecules released into the blood due to liver inflammation, molecules synthesized, regulated or excreted by the liver, and markers of processes commonly disrupted due to liver function impairment.[9]

Various indirect markers of liver fibrosis have been used in clinical practice for many years. For example the AST/ALT ratio, platelet count, AST/platelet ratio, prothrombin index etc.[9] Commonly used indirect markers are serum Alanine transaminase (ALT), Prothrombin time (PT) and Hepatitis B virus DNA load (HBV-DNA) to monitor CHB patients.

Frank cirrhosis and decompensated chronic liver disease can be diagnosed easily without liver biopsy. Patients with hepatic fibrosis in CHB patients are often asymptomatic with few clinical signs of liver disease. Recognition of presence of fibrosis is difficult without liver biopsy. Detection of hepatic fibrosis is not only for the diagnosis and prognosis of disease, but also for the good indicator to start or delay the antiviral treatment[10]. Therefore, to assess the prognosis and stages of hepatic fibrosis non-invasive markers are needed. So, in this study, correlation of serum ALT, PT and HBV-DNA with different stages of hepatic fibrosis were done to see their significance in diagnosis of hepatic fibrosis in CHB.

Methods

This cross sectional study was conducted at the Department of Clinical Pathology, in collaboration with Department of Hepatology and Department of Pathology, Bangabandhu Sheikh Mujib Medical University, Dhaka over the period from March' 2012 to February' 2013. 40 CHB patients who admitted in the Department of Hepatology were enrolled in the 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 in a

predesigned questionnaire. Serum ALT, PT and HBV-DNA reports were collected from patient's file. Needle liver biopsy was done by Hepatologist. Biopsy material was fixed in 10% formalin and sent to the Department of Pathology, BSMMU for complete histopathological examination. Only Haematoxyline and Eosin stain was done to see the hepatic fibrosis. It was done according to following Knodell's scoring system, Stage of fibrosis: F_0- no fibrosis, F_1-Fibrous portal expansion, F_3-Bridging fibrosis and F_4 –Cirrhosis.[11] All data was recorded systematically in a preformed data collection sheet and expressed as mean ± SD. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were find out from the test result. Statistical analyses of the results were obtained by Chi square test, ANOVA test and Spearman's rank correlation test. All statistical computations were performed by using window based computer software devised with Statistical Packages for Social Sciences (SPSS 17.0). Prior to the commencement of this study, the research protocol was approved by the Ethical Institutional Review Board of BSMMU, Dhaka.

Results

A total of 40 patients with CHB were included in this study. Maximum patients' age were belonged to 20-29 years in three groups. The mean age was found 30.25±10.86 years with range from 19 to 52 years in F_0 group, 27.14±7.65 years with range from 18 to 52 years in F_1 group and 28.45±9.05 years with range from 18 to 50 years in F_3 group. The mean age difference was not statistically significant (P>0.05) among three groups (Table I).

Regarding the gender distribution of the study patients, male were predominant in three groups, which was 5(62.5%) in F_0 group, 16(76.2%) in F_1 group and 11(100.0%) in F_3 group. The difference was not statistically significant (P>0.05) between two groups (Table II).

ALT was found 40.38±10.78 U/L in F_0 group, 44.67±15.01 U/L in F_1 group and 49.31±21.35 U/L in F_3 group. Mean PT was found 12.65±0.6 Sec. in F_0 group, 12.75±0.78 Sec. in F_1 group and 12.61±0.81 Sec. in F_3 group. Mean HBV-DNA was found 5.19±1.34 copies m/l in F_0 group, 5.23±2.13 copies m/l in F_1 group and 4.9±1.78 copies m/l in F_3 group. Knodell score difference was not statistically significant (P>0.05) among three groups (Table III).

ALT (>40U/L) was found 18(56.2%) in positive fibrosis and 4(50.0%) in negative fibrosis. PT (>14sec) was found 2(6.06%) in positive fibrosis and 1(14.28%) in negative fibrosis. HBV-DNA positive was found 12(37.5%) in positive fibrosis and 2(25.0%) in negative fibrosis (Table IV).

The validity of ALT, PT and DNA evaluation for fibrosis were correlated by calculating sensitivity, specificity, accuracy, positive and negative predictive values. Sensitivity of ALT, PT and DNA were 56.3%, 6.06 and 37.5%, specificity 50.0%, 85.7 and 75.0%, accuracy 55.0%, 0.2% and 45.0%, positive predictive value (PPV) were 81.8%, 66.6 and 85.7%, negative predictive value (NPV) were 22.2%, 16.2% and 23.1% respectively (Table V).

Serum ALT of 40 patients with CHB were expressed in U/L and Stages of fibrosis evaluated by histopathology was expressed in category. No correlation was found between ALT and fibrosis. The value of Spearman's rank correlation coefficient was 0.164, which is no correlation (p=0.321). Therefore, there was no linear correlation between ALT and fibrosis (Fig. 1).

PT of 40 patients with chronic hepatitis B was expressed in seconds and stages of fibrosis evaluated by histopathology was expressed in category. No correlation was found between PT and fibrosis. The value of Spearman's rank correlation coefficient was -0.087, which is no correlation (p=0.593). Therefore, there was no linear correlation between PT and fibrosis (Fig. 2).

HBV-DNA of 40 patients with chronic hepatitis B was expressed in copies m/l and Stages of fibrosis evaluated by histopathology was expressed in category. No correlation was found between HBV-DNA and fibrosis. The value of Spearman's rank correlation coefficient was -0.129, which is no correlation (p=0.428). Therefore, there was no linear correlation between HBV-DNA and fibrosis (Fig. 3).

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

	Stages of fibrosis						
Age in years	F0 (n=8)		F1 (n=21)		F3 (n=11)		
	n	%	N	%	n	%	
<20	1	12.5	2	9.5	1	9.1	P value* 0.687^{ns}
20-29	4	50.0	13	61.9	6	54.5	
30-39	1	12.5	5	23.8	3	27.3	
≥40	2	25.0	1	4.8	1	9.1	
Mean±SD	30.25±10.86		27.14±7.65		28.45±9.05		

ns=not significant

* ANOVA test was done to measure the level of significance.

F_0=no fibrosis, F_1=Fibrous portal expansion, F_3=Bridging fibrosis.

Table II: Distribution of the study patients by gender (n=40)

Sex	F0 (n=8)		F1 (n=21)		F3 (n=11)		
Male	5	62.5	16	76.2	11	100	*P* Value* 0.106^{ns}
Female	3	37.5	5	23.8	0	0	

ns=not significant

*Chi square test was done to measure the level of significance.

Table III: Mean ALT, PT and HBV-DNA of the study patients (n=40)

Variables	F0 (n=8)	F1 (n=21)	F3 (n=11)	*P* value
	Mean±SD	Mean±SD	Mean±SD	
ALT(U/L)	40.38±10.78	44.67±15.01	49.31±21.35	0.500[ns]
Range (min-max)	(29-64)	(26-73)	(16-76)	
PT (Sec)	12.65±0.6	12.75±0.78	12.61±0.81	0.871[ns]
Range (min-max)	(11.8-13.7)	(11.8-15)	(11.8-14.4)	
HBVDNA (copies m/l)	5.19±1.34	5.23±2.13	4.9±1.78	0.893[ns]
Range (min-max)	(3.77-8.08)	(1.35-12.18)	(2.96-9.34)	

s=significant; ns=not significant
P value reached from ANOVA test.

Table IV: Distribution of the study patients according to fibrosis with ALT, PT and HBV-DNA (n=40)

	Fibrosis			
Variable	Positive (n=32)		Negative (n=8)	
ALT	n	%	n	%
Positive (>40)	18	56.2	4	50.0
Negative (≤40)	14	43.8	4	50.0
PT				
Positive (≥56.6)	2	6.06	1	14.28
Negative (≤56.5)	31	93.93	6	85.71
DNA				
Positive	12	37.5	2	25.0
Negative	20	62.5	6	75.0

Table V: Sensitivity, specificity, accuracy, positive and negative predictive values of the ALT, PT and HBV-DNA evaluation for fibrosis

Validity test	ALT	PT	DNA
Sensitivity	56.3	6.06	37.5
Specificity	50.0	85.7	75.0
Accuracy	55.0	0.2	45.0
Positive predictive value	81.8	66.6	85.7
Negative predictive value	22.2	16.2	23.1

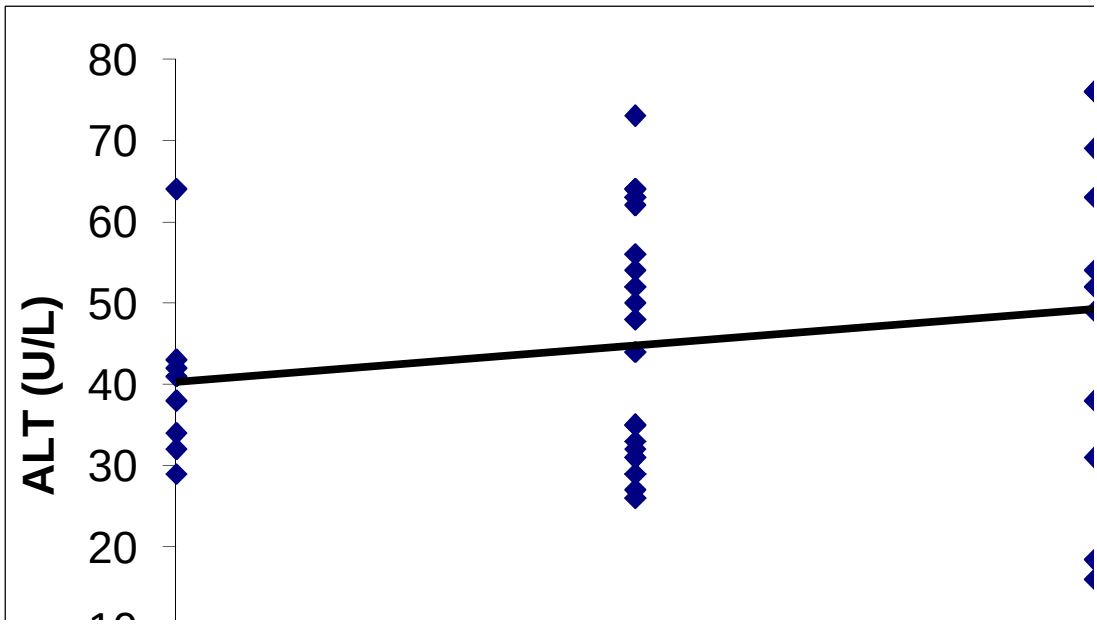

Fig 1. Scatter diagram showing no correlation (r=0.164;p=0.312) between ALT and fibrosis

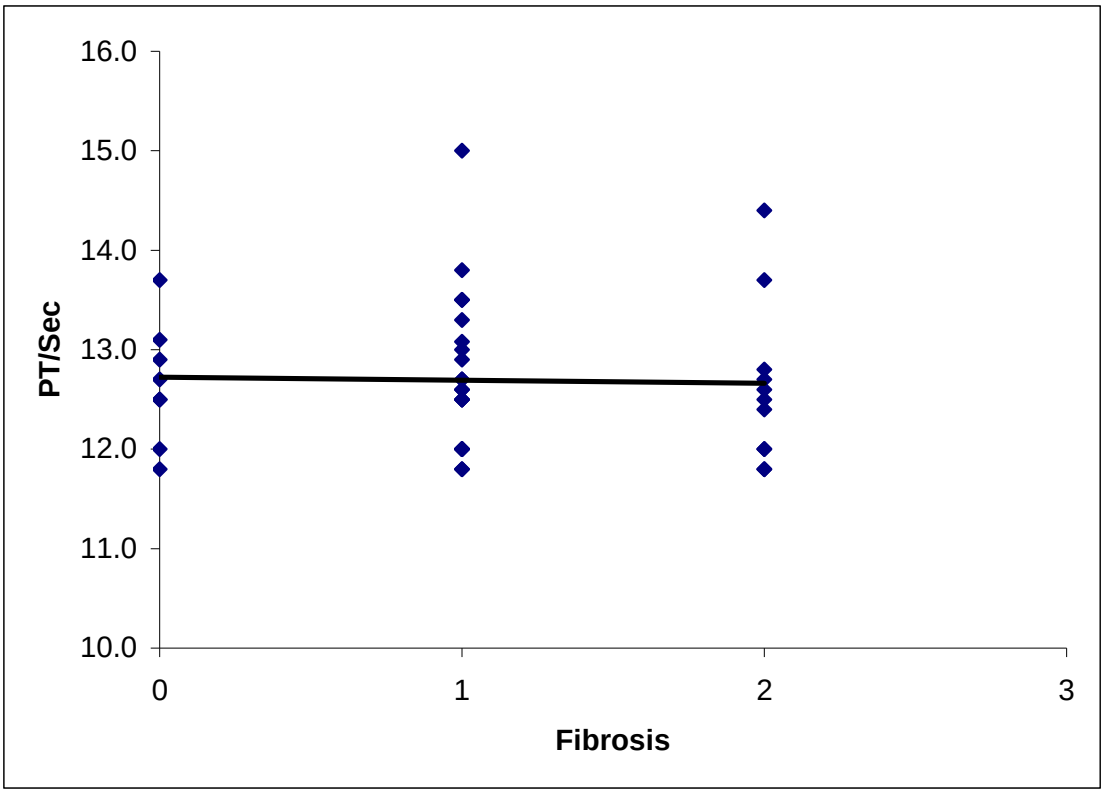

Fig 2. Scatter diagram showing no correlation (r=-0.087;p=0.593) between PT and fibrosis

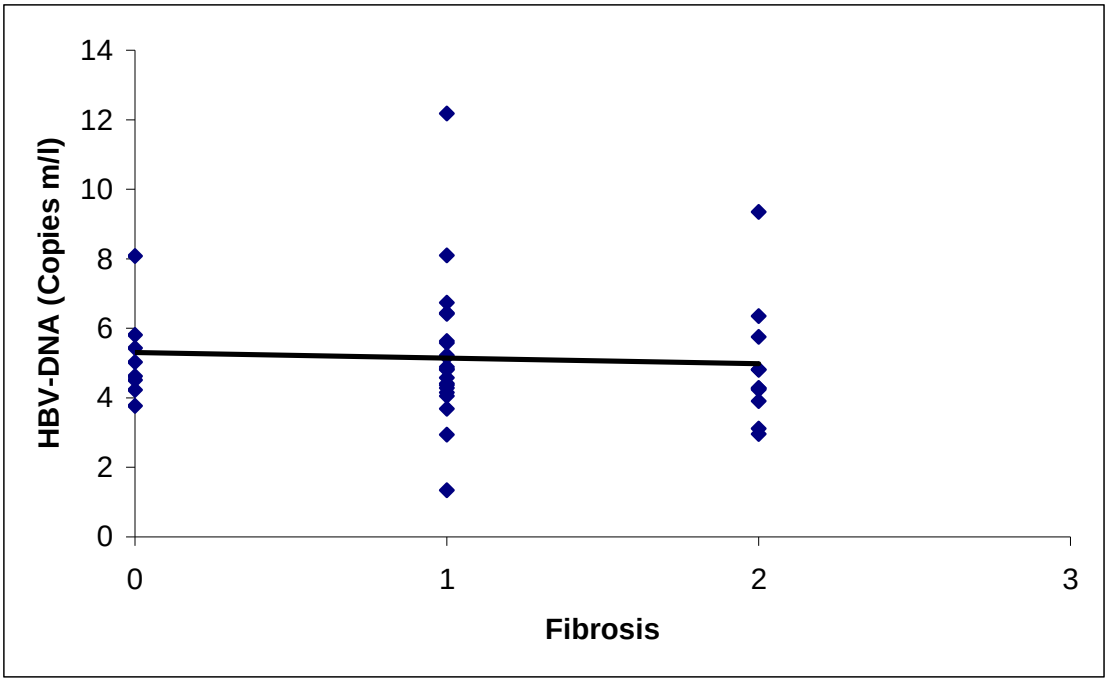

Fig 3. Scatter diagram showing no correlation (r=-0.129; p=0.428) between HBV-DNA and fibrosis

Discussion

Non-invasive indirect markers of liver fibrosis have been used in clinical practice for many years. In this present study patients were divided into 4 groups based on fibrosis stage, including F_0, F_1, F_3 and F_4.[11] Masson's-Trichome stain needed to identify hepatic fibrosis which was not done in this study.

A total of 40 patients with CHB were included in this study. Maximum patients' age were belonged to 20-29 years in three groups. The mean age was found 30.25±10.86 years with range from 19 to 52 years in F_0 group, 27.14±7.65 years with range from 18 to 52 years in F_1 group and 28.45±9.05 years with range from 18 to 50 years in F_3 group. Alam et al., (2008) found that chronic hepatitis B affects the younger population (age group 21-30) of Bangladesh.[12] This finding was similar with our study.[12]

Regarding the gender distribution of the study patients, male were predominant in three groups, which was 5 (62.5%) in F_0 group, 16 (76.2%) in F_1 group and 11 (100.0%) in F_3 group. The difference was not statistically significant (P>0.05) between two groups. Rahman[3] (2011) observed that males were predominant in CHB patients in Bangladesh which was consistent with our study.

The validity of ALT, PT and HBV-DNA evaluation for fibrosis were obtained by calculating sensitivity, specificity, accuracy, PPV and NPV. Sensitivity of ALT, PT and HBV-DNA were 56.3%, 6.06 and 37.5%, specificity 50.0%, 85.7 and 75.0%, accuracy 55.0%, 0.2 and 45.0%, PPV were 81.8%, 66.6 and 85.7%, and NPV were 22.2%, 16.2% and 23.1% respectively. We also performed correlation between hepatic fibrosis with ALT, PT and HBV-DNA levels. The values of spearman's rank correlation coefficient were 0.164, -0.087 and -0.129 respectively which suggested no correlation. Our finding was equal to previous study.[13,14,15]

According to our study findings, serum ALT, PT and HBV-DNA not correlated with the

stages of hepatic fibrosis. Our data indicates that serum ALT, PT and HBV-DNA did not exhibit good diagnostic performance and are not useful for a guideline for presenting fibrosis.

Conclusion

Our study revealed that serum ALT, PT and HBV-DNA are not significantly higher in patients with CHB. Validity tests of these markers are also not significant. There was no statistically significant correlation between serum ALT, PT and HBV-DNA levels with different stages of hepatic fibrosis. We conclude that serum non-invasive indirect markers like serum ALT, PT and HBV-DNA levels are not significant to use for the diagnosis of hepatic fibrosis in CHB.

References

1. Lok A F, McMahon BJ, Chronic hepatitis B: AASLD Practice Guideline. Hepatology, 2007;45(2)507-539.
2. Boyer TD, Manns MP, SanylA J, 2012. Zakim and Boyers hepatology, 6th edition. Saunders Elsevier Inc.
3. Rahman S, 'HepatitisB; From Blumberg to Bangladesh', Euroasian journal of Hepatogastroentrology, 2011; 1 (2): 42-43.
4. Mahtab M A, Rahman S, Karim F, Khan M, Foster G, Solaiman S, Afroz S. Epidemiology of hepatitis B virus in Bangladeshi general population. Hepatobiliary Pancreatic Int. 2008 ; 7: 595-600.
5. Griinhage F, LammertF. 2012, Hepatology, A clinical textbook. In: Mauss, B., Rockstroh., Serraz, W., Mauss, B., Rockstroh., Serraz, W., (eda) 3rd edition., Germany: Flying publisher,PP.326-334.
6. Duncan MR, Frazier KS, Abramson S. Connective tissuegrowth factor mediates transforming growth factor beta-induced collagen synthesis: Down-regulation by camp. FASEB J. 1999;13(13):1774–1786.
7. Braticevici C A, Purcarea M. Non - biopsy methods to determine hepatic fibrosis, Journal of Medicine and Life. 2009; 2 :(4), 401-406
8. Bedossa P, Dargere D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. Hepatology, 2003.38:1449-1457.
9. Afdhal NH, Nunes D, 2004, 'Evaluation of liver fibrosis: A concise review', American Journal of Gastroenterol, vol. 99, no. 6; pp. 1160–1174.
10. Grigorescu M, 'Non invasive Biomedical Markers of Liver Fibrosis'. Journal Gastrointestineand Liver Dis, 2006; 15: 149-159.
11. Knodell RG, Ishak KG, Black WC, Chen TS, Craig R, Kaplowilowitz N, 'Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis', Hepatology, 1981; q: 431-435.
12. Alam S, Ahmad N, Mustafa G, Alam K, Khan M, 'Characteristics of treatment naive chronic hepatitis B in Bangladesh: Younger population are more affected; HBeAg negatives are more advanced', Saudi journal of gastroenterology, 2008; 14 (1): 15-18.
13. Alam S, Ahmad N, Mustafa G, Alam K, Khan M, 2008, 'Correlation between Hepatitis B viral load and extent of liver pathology in patients with chronic hepatitis B', Hepatitis monthly, vol. 8; pp. 185-189.
14. Haafiz, A., Farrington, C., Andres, J., Islam, S., 2011. Relationship between hepatic CTGF expression and routine blood tests at the time of liver transplantation for billiarry atreasia: hope or hype for a biomarker of hepatic fibrosis, Clinical and Experimental Gastroenterology, 4: 49-54.
15. Seto WK, Lai CL, Fung J, Wong D, 2012, 'A Large Population Histology Study Showing the Lack of Association between ALT Elevation and Significant Fibrosis in Chronic Hepatitis B',. PLoS ONE, vol. 7, no. 2; pp. e32622