“Correlation between Serum Hyaluronic Acid Level and Liver Fibrosis in Patients with Chronic Hepatitis B: A Study in Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh”


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Abstract

Introduction: Bangladesh is an intermediate endemic country for hepatitis B, with a huge burden of CHB patients. Early diagnosis and treatment can reduced the adverse sequelae of CHB. The aim of the study was carried out to evaluate the serum hyaluronic acid level as a non-invasive marker of hepatic fibrosis in chronic hepatitis B. Objective: To find out correlation between serum hyaluronic acid level and liver fibrosis in patients with chronic hepatitis B. Methods: Enrolled patients for study were divided into two groups, Group-1, 40 patients with chronic hepatitis B. Group 2, thirty age and sex matched patients without liver disease was served as control. Study was done in the department of Hepatology, Bangabandhu Sheikh Mujib Medical University, Dhaka, during the period of July 2007 to June 2009. We included patients with chronic hepatitis B characterized by, HBsAg positive for more than 6 months, HBeAg positive or negative, serum HBV DNA in PCR (more than 107 copies/ml in HBeAg positive or more than 106 copies/ml in HBeAg negative case), serum ALT normal or elevated and excluded the patients who were decompensated cirrhosis, co-infection with hepatitis C virus, with history of alcoholism, with anti HBV therapy, drug induced hepatitis, sonologic evidence of NAFLD and patients with rheumatological disorder. 5 ml of peripheral blood was taken from each subject in tasting state in complete rest for measurement of serum hyaluronic acid by using the Corgenix HA Test Kit. Liver biopsy was done at group-1 for complete histopathological examination including HAI and Knodell score. Results: Mean age of the healthy subjects was (26.6±6.37) years and of the patients was a (27.67±6.85) years. There were 33% patients were minimal necro inflammation (HAM-3), 50% were mild (HA! =4-8), 17% were moderate (HAI= 9-12). Among the patients stage -1 fibrosis was 56.7% and stage -3 was 43.3%. in our study, Serum HA in CHB was (Mean ± SD), 70.3 ± 42.7 ng/ml and control patients was (Mean ± SD), 18.3 ± 9.0 ng/ml. Mean level of HA in different grades of histological activity index are shown that HA level is higher in moderate HAI score than mild and minimal. We found there was positive correlation between hyaluronic acid and HAI score (P < 0.005). So this test can be done to detect and assess the severity of different grades of histological activity. The mean HA level in stage -1 fibrosis is 38.5 ng/ml and stage -3 fibrosis is 111.95 ng/ml. We found there was highly significant correlation between hyaluronic acid and fibrosis (P < 0.001). Conclusion: There was strong positive correlation between serum hyaluronic acid levels and degree of liver fibrosis Serum hyaluronic acid can be used for assessment of liver fibrosis in patients of chronic hepatitis B during diagnosis and follow up. So it is concluded that hyaluronic acid may be a useful marker of hepatic fibrosis as well as histological activity.

Keywords: Hepatitis B virus, serum hyaluronic acid, liver fibrosis, chronic hepatitis B.

INTRODUCTION

Hepatitis B virus (HBV) infection is a global public health problem. It is currently estimated that 400 million people worldwide have chronic HBV infection [1]. Chronic Hepatitis B (CHB) may be defined as chronic necro-inflammatory disease of the liver caused by persistent infection with hepatitis B virus [2].
Chronic Hepatitis B can be subdivided into HBeAg positive and HBeAg negative CHB. It causes a spectrum of different diseases ranging from clinically asymptomatic carrier state to the development of cirrhosis and hepatocellular carcinoma (HCC)[3]. Chronic HBV infection is responsible for 500,000 to 1.2 million deaths every year, and is the 10th leading cause of death worldwide [4]. It is particularly important in the Asian Pacific region where the prevalence is high [5]. In this part of the world, the majority of HBV infection is acquired perinatally or in early childhood. HBV infection is also a common problem in our country mainly affects the younger population and lead to the development of cirrhosis and HCC at younger ages [6, 7]. Liver biopsy has been the gold standard to evaluate the histological stage of liver fibrosis and an integral part of management of chronic hepatitis B; but the procedure is invasive, costly and carries a definite risk of occasional complications such as post procedure pain, haemorrhage and even death[8]. Hence, several non-invasive serological markers have been reported to predict the presence of significant fibrosis or cirrhosis in patients with CHB, among them AST/ALT ratio, AST to platelet ratio index (APRI), and age platelet count index (API) are based on routine laboratory results [9, 10]. Recently several clinical studies have attempted to identify serum markers like hyaluronic acid, procollagen-III, collagen-IV, matrix metalloproteinases, tissue inhibitor metalloproteinases and imaging techniques like fibroscan that correlate with the degree of cirrhosis or fibrosis and thus could be used in conjunction or in place of a liver biopsy. Among these hyaluronic acid is particular interest [11, 12]. Hyaluronic acid is an unbranched high molecular weight polysaccharide that is widely distributed in the extracellular spaces. Part of the hyaluronic acid enters the general circulation via the lymphatic system and is rapidly cleared and degraded mainly in the hepatic sinusoidal endothelial cells [13, 14]. A fibrotic liver shows both relative and absolute increase in hyaluronic acid. Circulating HA measurement may be helpful in differentiating non-cirrhotic from cirrhotic liver, for monitoring liver function and for evaluating the extent of liver fibrosis [15].

**OBJECTIVE**

To find out Correlation between serum hyaluronic acid level and liver fibrosis in patients with chronic hepatitis B

**METHODS**

Enrolled patients for study were divided into two groups, Group-1, forty patients with chronic hepatitis B aged 18 to 50 years. Group-2, thirty age and sex matched patients without liver disease was served as control. Study was done in the department of Hepatology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, during the period of July 2007 to June 2009. We included patients with chronic hepatitis B characterized by, HBsAg positive for more than 6 months, HBeAg positive or negative, serum HBV DNA in PCR (more than 10^5 copies/ml in HBeAg positive or more than 10^6 copies/ml in HBeAg negative case), serum ALT normal or elevated. We were excluded the patients who were decompensated cirrhosis, co-infection with hepatitis C virus, with history of alcoholism, with anti HBV therapy, drug induced hepatitis, sonologic evidence of NAFLD and patients with rheumatological disorder. For group-1, patients presenting with HBSAg-positive was evaluated by thorough history and physical examination. Suggestive patients were underwent serum ALT, HBeAg, Anti HBe, HBV-DNA (PCR) to diagnose chronic hepatitis B and others investigations like Anti HCV, USG of hepatobiliary system to fulfill exclusion criteria. For group-2, patients without clinical, biochemical, serological evidence of liver disease, such as patients of irritable bowel syndrome, peptic ulcer disease was taken and evaluated by thorough history and physical examination. Suggestive patients were underwent serum ALT, HBsAg, AntiHCV, USG of hepatobiliary system to exclude liver disease. After having consent 5 ml of peripheral blood was be taken from each patient of group-1 and group-2 in fasting state in complete rest and send to Dept. of Microbiology and Immunology, BSMMU for measurement of serum hyaluronic acid by using the Corgenix HA Test Kit. Each patient of group-1 was admitted in hospital and after informed written consent from the patient liver biopsy was done and the specimens sent to the department of pathology, BSMMU for complete histopathological examination including HAI and Knodell score without any clinical, biochemical, serological and virological information of the patients. All collected data was analyzed by SPSS version 10. Values were expressed either as mean ± SD or in frequency or in percentages. Comparison between groups was done by Chi-square test, student T test and ANOVA test. Pearson test was used for correlation analysis and p value of <0.05 was considered to be statistically significant.

**RESULTS**

The age range of the healthy subjects was 18-50 years and mean age was (26.6±6.37) years. The age range of the patients was 18-50 years and the mean age was (27.67±6.85) years. The highest incidence of CHB as well as control was found at 20-29 age groups. A male preponderance was observed in 73.3% the study population (n=70). Their sex matched in both case and control. Biochemical profile of CHB patients is shown the range of ALT was 19-103 (46.7±22.38) U/L and AST was 15-130 (44.30±26.65) U/L the range of prothrombin time was 12-16 (13.97±1.25) sec, platelets count range was (160-450) x10 9/L. Among the patients HBeAg positive was 23.3% and HBeAg negative was 76.7% and Anti HBe positive was 70% and Anti HBe negative was 30%. Mean level of HA in different grades of histological activity index are shown that HA
level is higher in moderate HAI score than mild and minimal.

### Table-1: Correlation between noninvasive markers and fibrosis (N=40)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean±SD</th>
<th>r-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA</td>
<td>70.34±42.75</td>
<td>0.866</td>
<td>0.000</td>
</tr>
<tr>
<td>ALT</td>
<td>40.85±18.73</td>
<td>0.208</td>
<td>0.271</td>
</tr>
<tr>
<td>AST</td>
<td>44.3±26.65</td>
<td>0.154</td>
<td>0.416</td>
</tr>
<tr>
<td>APRI</td>
<td>0.50±0.41</td>
<td>0.103</td>
<td>0.587</td>
</tr>
<tr>
<td>PT</td>
<td>13.9±1.2</td>
<td>0.126</td>
<td>0.507</td>
</tr>
<tr>
<td>Platelets count</td>
<td>263.33±66.14</td>
<td>0.059</td>
<td>0.758</td>
</tr>
<tr>
<td>DNA (log)</td>
<td>5.99±2.3</td>
<td>-0.113</td>
<td>0.552</td>
</tr>
</tbody>
</table>

### Table-2: Summaries of HA Level in both group of patients (N=40)

<table>
<thead>
<tr>
<th>Group</th>
<th>HA Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHB (ng/ml)</td>
<td>Control (ng/ml)</td>
</tr>
<tr>
<td>Mean</td>
<td>70.3</td>
</tr>
<tr>
<td>Minimum</td>
<td>20.5</td>
</tr>
<tr>
<td>Maximum</td>
<td>174.2</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>42.7</td>
</tr>
<tr>
<td>No</td>
<td>40</td>
</tr>
</tbody>
</table>

### Table-3: HA Level in different stages of fibrosis (n=70)

<table>
<thead>
<tr>
<th>Stages of fibrosis</th>
<th>Hyaluronic acid Mean ± SD</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>18.3 ± 9.020</td>
<td>3.6</td>
<td>46.5</td>
</tr>
<tr>
<td>1</td>
<td>38.53 ± 13.613</td>
<td>20.5</td>
<td>62.5</td>
</tr>
<tr>
<td>3</td>
<td>111.95 ± 29.344</td>
<td>56.2</td>
<td>174.2</td>
</tr>
<tr>
<td>Total</td>
<td>44.33 ± 40.333</td>
<td>3.6</td>
<td>174.2</td>
</tr>
</tbody>
</table>

### DISCUSSION

Bangladesh is an intermediate endemic country for hepatitis B, with a huge burden of CHB patients, 7.2%-7.5% of population [16,17]. Early diagnosis and treatment can reduced the adverse sequelae of CHB. The study was carried out to assess usefulness of hyaluronic acid for assessing liver fibrosis in CHB patients. Serum hyaluronic acid estimation is a cheap, simple-to-perform, minimally invasive, not widely available, needs baseline tests, transfusion facilities, trained personnel for biopsy, follow-ups and pathological support with competent pathologist. Many patients are reluctant to undergo the invasive procedure. The CHB patients were included without having any condition like any rheumatological diseases, renal diseases, antiviral drugs therapy that may cause changes in serum HA. The age range of the patients was 18-50.
years and the mean age was (27.67±6.85) years. The highest incidence, 64% of CHB patients were found at 20-29 age groups which were similar to previous study done among our people [18, 19]. In this study, among CHB patients male were 73.3% and female were 26.7%. This male preponderance 73.3% was observed in the study population which was similar in previous study done among Bangladeshi general population [5, 19]. There were age and sex matched in both case and control. Among the patients HBeAg positive was 23.3% and HBeAg negative was 76.7% and Anti HBe positive was 70% and Anti HBe negative was 30%. Predominance of HBeAg negative CHB patients was also similar in previous study done in Bangladesh [6, 17, 18]. Among the patients stage-1 fibrosis was 56.7% and stage-3 was 43.3%. No patient in stage 2 because the histopathological examination was done in Knodell scoring system in which stage 2 fibrosis was absent. As cirrhosis was clinically excluded from the study, so stage 4 was absent in this study, even after that histological stage-4 fibrosis would be possible but incidentally this stage-4 fibrosis or cirrhosis was not found in any patient. The correlation between fibrosis with HA, ALT, AST, APRI, PT, platelets count and DNA count were done. The P value found that none of them significantly correlate with fibrosis except HA. In this study serum HBV DNA levels did not correlate with the fibrosis which was similar in previous study [1, 18, 19]. As a result hyaluronic acid is one of the best noninvasive makers for hepatic fibrosis. Mean level of HA in different grades of histological activity index are shown that HA level is higher in moderate HAI score that mild and minimal. We found there was positive correlation between hyaluronic acid and HAI score (r = 0.496, P < 0.005) which was similar with previous study [15, 21]. So this test can be done to detect and assess the severity of different grades of histological activity. In our study, Serum HA in CHB was (Mean ± SD), 18.3 ± 9.0 ng/ml, minimum HA level was 3.6 g/ml and maximum 46.5 g/ml. Among CHB patients 40% had HA levels more than 75 ng/ml, 30% more than 100ng/ml. The mean HA level in stage-1 fibrosis is 38.5 ng/ml and stage-3 fibrosis is 111.95 ng/ml which was related so previous study. We found there was highly significant correlation between hyaluronic acid and fibrosis (P < 0.001) [15, 20-24]. Thus the results of our study were comparable with those of other study done earlier in different countries. So serum HA levels can be used for predicting the degree of fibrosis and also excluding significant fibrosis, which is very important especially in countries with high numbers of hepatitis patients. This study had several limitations. Sample size was small the number, we could not differentiate all fibrosis stages as Metavir scoring and Masson’s-Trichrome stain was not done. The ALT levels in our study patients were below two times than normal. Further large scale cohort study is recommended to validate these results.

**Conclusion**

The findings obtained from this study suggest that serum hyaluronic acid is a useful noninvasive market of liver fibrosis. There was strong positive correlation between serum hyaluronic acid levels and degree of liver fibrosis under the study conditions. Serum hyaluronic acid can be used for assessment of liver fibrosis in patients of chronic hepatitis B during diagnosis and follow up. So it is concluded that hyaluronic acid may be a useful market of hepatic fibrosis as well as histological activity.

**Reference**

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