Research Article

Arzu Şenol*, Şafak Özer Balin and Zülal Aşçı Toraman

Fetuin A and fetuin B as an indicator of liver fibrosis in hepatitis B

https://doi.org/10.1515/tjb-2022-0197
Received October 2, 2022; accepted May 9, 2023; published online October 10, 2023

Abstract

Objectives: The aim of this study is to investigate the diagnostic and prognostic characteristics of fetuin A and fetuin B in chronic hepatitis B (CHB) and HBe Ag-negative chronic infection (HCI) and the relationship between the levels of these proteins and fibrosis in CHB.

Methods: In this study, we examined 98 patients with CHB, 58 with HCI and 42 control groups. Fetuin A and B levels were determined via ELISA.

Results: Serum fetuin A and B levels were significantly higher in the control group than the hepatitis B cases (p=0.001). No significant difference in fetuin A and B levels between patients in the CHB and HCI. In the CHB, fetuin A level was significantly lower in patients with significant fibrosis than those with mild fibrosis (p=0.007). Fetuin B was lower in patients with significant fibrosis than in with mild fibrosis; however, this difference was not significant. In predicting the absence of significant fibrosis, the area under the curve was estimated as 0.855 for fetuin A and 0.866 for fetuin B using the ROC curve.

Conclusions: Fetuin A and B were lower in CHB and HCI compared to the control group and there was no difference between the two groups suggests that these proteins may be effective in the pathogenesis of hepatitis B-induced liver damage. Fetuin A and B, which are found to be lower in patients with significant fibrosis in CHB, can be used as non-invasive markers in the early detection of fibrosis and in the follow-up of progression to significant fibrosis.

Keywords: hepatitis B; fibrosis; fetuin A; fetuin B; ELISA

Introduction

Hepatitis B viruses (HBVs) are the causative agents of liver cirrhosis (LC) and hepatocellular carcinoma (HCC) [1, 2]. Identification of specific and sensitive biomarkers for liver diseases is of paramount importance. Serum glycoproteins are considered potential biomarkers in combination with other clinical markers [1]. Hepatokines are proteins that have been shown to be important regulators of biological processes [3]. Fetuins are members of the cystatin family and are multifunctional glycoproteins primarily secreted by approximately 95% of human hepatocytes. The fetuin family of proteins includes fetuin A and fetuin B, among others [2, 4].

Few studies have examined fetuin A levels in patients with liver diseases, and fetuin A levels are thought to predict the prognosis of these patients or reflect parenchymal cell damage in the liver [5]. Serum concentration of fetuin A, a negative acute phase protein, is reported to be a good indicator of liver function and mortality [1]. Hepatic secretion of fetuin A is negatively regulated by several pro-inflammatory cytokines, and fetuin A administration provides dose-dependent and long-lasting protection against systemic inflammatory diseases [6]. Fetuin B is the second member of the fetuin protein family and is involved in metabolic regulation. Although fetuin A and fetuin B are different in terms of gene regulation, they have partially similar functions [7]. Our knowledge on fetuin B is limited [8].

The aim of this study is to investigate the diagnostic and prognostic characteristics of fetuin A and fetuin B in chronic hepatitis B (CHB) and HBe Ag-negative chronic infection (HCCI inactive HBS Ag carrier) and the relationship between the levels of these proteins and fibrosis in CHB. The results from this study will help us better understand the role of fetuin A and fetuin B in the pathogenesis of CHB and HCCI, which will assist clinicians in predicting disease progression and planning effective treatment.
Materials and methods

In this prospective study, we examined 58 with HCl (≥6 months HBs Ag positive, HBe Ag negative, Anti HBC IgG positive, HBV DNA <31.6 IU/mL) and 98 patients with CHB (≥6 months HBs Ag positive, Anti HBC IgG positive, HBe Ag negative/positive, HBV DNA of >2000–20,000 IU/mL) who applied to the Infectious Diseases outpatient clinic between July 2017 and March 2019 and underwent liver fine needle aspiration biopsy. In addition, 42 controls without any history of acute and chronic hepatitis or any chronic disease were included.

The diagnosis of chronic HBV and HCl was made according to the European Association for the Study of the Liver criteria [9]. Demographic data of the patients were obtained from electronic patient records. The exclusion criteria of this study were as follows: LC; HCC; Hepatitis B surface antigen (HBsAg), HBV viral load (HBV DNA), HBe-Ag negative chronic infection; M, male; F, female. Gender, F/M ratio were 50:48 for patients with CHB, 46.76±2.38 years and 27.31 for those with HCl, and 37.00±3.46 years and 20.22 for those in the control group, respectively. No significant intergroup difference was noted in terms of age, sex, AST, ALT, total cholesterol, TG, and PTZ values (p>0.05). Serum fetuin A and fetuin B levels were significantly higher in the control group than the CHB and HCl groups (p<0.001). No significant difference in fetuin A and fetuin B levels between patients in the CHB and HCl groups (p>0.05). Demographic characteristics, mean values of laboratory tests, and fetuin A and fetuin B levels of CHB patients, HCl patients, and the control group are summarized in Table 1.

No significant difference was found between CHB patients, HCl patients, and the control group in terms of fetuin A levels, AST, ALT, AFP, and albumin levels (p>0.05). However, significant differences were observed in fetuin B levels and AST, ALT, AFP, and albumin levels (p<0.001). No significant intergroup difference was observed in fetuin A and fetuin B levels, cholesterol, and triglyceride levels (p>0.05).

In the CHB group, serum fetuin A level was significantly lower in patients with significant fibrosis than in patients with mild fibrosis (p=0.007). Serum concentration of fetuin B

Table 1: Demographic data of hepatitis B patients and control group, average laboratory values.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CHB (n: 58)</th>
<th>HCl (n: 58)</th>
<th>Control (n: 42)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>43.8±14.2</td>
<td>45.9±9.5</td>
<td>38.7±7.75</td>
<td>0.108</td>
</tr>
<tr>
<td>Gender, F/M</td>
<td>50/48</td>
<td>27/31</td>
<td>20/22</td>
<td>0.521</td>
</tr>
<tr>
<td>AST, IU/L</td>
<td>63.57±12.21</td>
<td>30.68±1.19</td>
<td>25.57±1.17</td>
<td>0.135</td>
</tr>
<tr>
<td>ALT, IU/L</td>
<td>102.94±21.31</td>
<td>31.21±1.36</td>
<td>27.86±1.41</td>
<td>0.070</td>
</tr>
<tr>
<td>T BIL, mg/dL</td>
<td>0.63±0.06</td>
<td>1.28±0.64</td>
<td>0.35±0.04</td>
<td>0.096</td>
</tr>
<tr>
<td>Platelet, 10^9/L</td>
<td>270.62±11.71</td>
<td>250.33±12.55</td>
<td>229.43±10.33</td>
<td>0.251</td>
</tr>
<tr>
<td>AFP, µg/L</td>
<td>3.40±0.57</td>
<td>2.80±0.21</td>
<td>2.76±0.37</td>
<td>0.623</td>
</tr>
<tr>
<td>Albumin, g/dL</td>
<td>4.34±0.07</td>
<td>4.06±0.16</td>
<td>4.33±0.09</td>
<td>0.090</td>
</tr>
<tr>
<td>PTZ, s</td>
<td>1.26±0.47</td>
<td>1.02±0.21</td>
<td>1.16±0.15</td>
<td>0.139</td>
</tr>
<tr>
<td>T cholesterol, mg/dL</td>
<td>179.18±12.10</td>
<td>180.0±23.41</td>
<td>147.43±5.54</td>
<td>0.399</td>
</tr>
<tr>
<td>Triglyceride, mg/dL</td>
<td>137.90±19.09</td>
<td>146.50±16.78</td>
<td>73.57±3.40</td>
<td>0.081</td>
</tr>
<tr>
<td>Fetuin A, ng/mL</td>
<td>2.17±0.15</td>
<td>1.18±0.15</td>
<td>5.11±1.35</td>
<td>0.001</td>
</tr>
<tr>
<td>Fetuin B, ng/mL</td>
<td>108.17±46</td>
<td>127.59±10.50</td>
<td>122.4±147.81</td>
<td>0.001</td>
</tr>
</tbody>
</table>
| AST, aspartate aminotransferase; ALT, alanine aminotransferase; TBIL, total bilirubin; AFP, alpha fetoprotein; PTZ, prothrombin time; CHB, chronic hepatitis B; HCl, HBe-Ag negative chronic infection; M, male; F, female. Fetuin A and fetuin B levels; ***p<0.001, p=0.840 between the CHB-HCl group, p=0.001 between the CHB-control group and p=0.001 between the HCl-control group, respectively. One-Way ANOVA-Tukey.
Table 2: Laboratory parameters and fetuin A–B levels according to fibrosis stage in CHB cases.

<table>
<thead>
<tr>
<th></th>
<th>Mild fibrosis (stage 1–2) (n: 68)</th>
<th>Significant fibrosis (stage 3–4) (n: 30)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>41.93±17</td>
<td>46.85±2.69</td>
<td>0.219</td>
</tr>
<tr>
<td>Gender, M/F</td>
<td>33/35</td>
<td>16/14</td>
<td>0.902</td>
</tr>
<tr>
<td>Fetuin A, ng/mL</td>
<td>1.73±1.24</td>
<td>0.71±0.16</td>
<td>0.007</td>
</tr>
<tr>
<td>Fetuin B, ng/mL</td>
<td>115.21±10.5</td>
<td>94.98±5.95</td>
<td>0.089</td>
</tr>
<tr>
<td>AST, IU/L</td>
<td>50.03±6.24</td>
<td>71.0±28.83</td>
<td>0.329</td>
</tr>
<tr>
<td>ALT, IU/L</td>
<td>87.66±16.06</td>
<td>112.85±55.0</td>
<td>0.566</td>
</tr>
<tr>
<td>AFP, μg/L</td>
<td>2.73±0.14</td>
<td>5.20±2.19</td>
<td>0.091</td>
</tr>
<tr>
<td>HBV DNA, 10^3 IU/mL</td>
<td>64,248±37,934</td>
<td>12,219±8,802</td>
<td>0.370</td>
</tr>
</tbody>
</table>

AST, aspartate aminotransferase; ALT, alanine aminotransferase; AFP, alpha fetoprotein; M, male; F, female.

was lower in patients with significant fibrosis than in patients with mild fibrosis; however, this difference was not statistically significant (p<0.05).

In the CHB group, no significant difference was noted between patients with significant fibrosis and those with mild fibrosis in terms of age, sex, AST, ALT, AFP, and HBV DNA (p>0.05). Age, sex, AST, ALT, AFP, HBV DNA, and serum concentrations of fetuin A and fetuin B levels according to the degree of fibrosis in patients with CHB are shown in Table 2.

In predicting the absence of significant fibrosis in CHB patients, the area under the ROC curve was estimated as 0.855 for fetuin A and 0.866 for fetuin B (confidence interval 0.745–0.966 for fetuin A, 0.734–0.998 for fetuin B) using the ROC curve, whereas the cut-off value was 0.79 ng/mL for fetuin A (72% sensitivity and 84% specificity) and 99.3 ng/mL for fetuin B (82% sensitivity and 84% specificity) based on the results of the likelihood ratio test (Figure 1).

Discussion

A significant proportion of the world’s population is exposed to HBV at some point in their lives [11]. Although the exact mechanism of regulation of hepatic fetuin A secretion remains unknown, a relationship between fetuin A secretion and lipid metabolism has been demonstrated in the past [12]. Fetuin A secreted by healthy hepatocytes was reportedly affected by chronic liver disease and decreased with disease progression and aging. Moreover, in the same study, a significant difference was observed between serum concentration of fetuin A and that of TG and LDL-cholesterol [2].

Ix et al. [13] reported that fetuin A levels were higher in patients with high serum TG and LDL-cholesterol concentration. In the present study, no significant difference was observed between the CHB and HCI groups and the control group in terms of mean age. Cholesterol and TG values were higher in CHB and HCI cases than in the control group; however, this difference was not significant (p>0.05).

In another study, low serum fetuin A levels were reported in patients with CHB [12]. Dai et al. [14] also found low serum fetuin A levels in their study on patients with CHB. Several studies reported varying levels of fetuin A in patients with liver disease, indicating that it is a prognostic marker for liver injury and CHB [15, 16].

Fetuin A has been reported to be a negative acute phase reactant for inflammation, infection, and malignancy [17, 18]. Ma et al. [19] showed that CHB patients have low serum fetuin A levels, and that fetuin A levels are important in the development of seroconversion. Another study showed a significant decrease in serum fetuin A levels in patients with acute–chronic liver failure compared with patients with CHB and healthy controls. In addition, the serum concentrations of fetuin A were reportedly lower in CHB cases than in healthy controls [7, 14].

In one study, serum fetuin A levels were lower in individuals with elevated levels of liver enzymes, and serum fetuin A levels were reported to be important in showing the severity of liver damage [14]. In the present study, no significant difference was found in fetuin A levels with respect to AST, ALT, AFP, total bilirubin, and albumin levels in patients with CHB and HCI and individuals in the control group (p>0.05). However, a significant difference was observed in fetuin B levels with respect to AST, ALT, AFP, total bilirubin, and albumin levels (p<0.05). Another study reported that fetuin A levels were low in advanced stages of fibrosis and cirrhosis. In addition, the authors reported that decreased serum fetuin A levels may reflect critical liver injury and impaired liver function [5].

Yılmaz et al. [15] reported the absence of a significant decrease in serum fetuin A levels in patients with advanced fibrosis; however, they reported that it is important to determine fetuin A levels to show liver injury and fibrosis. In a study conducted in patients with severe liver damage such as chronic hepatitis, cirrhosis, and cancer, a decrease in serum fetuin A levels that was especially prominent in patients with advanced liver failure was identified and this was directly related to decrease in fetuin A secretion in the liver. A significant decrease in serum fetuin A levels was observed from F0 (no fibrosis) to F4 (cirrhosis). These findings suggest that serum fetuin A level is directly linked to the severity of liver injury and may be an indicator of liver cell function and poor prognosis [7].

In the present study, serum fetuin A levels were significantly lower in patients with significant fibrosis than in
patients with mild fibrosis (p=0.007). Similarly, fetuin B levels were lower in patients with significant fibrosis than in those with mild fibrosis. However, this difference was not statistically significant (p>0.05). In addition, no significant difference in terms of age, sex, AST, ALT, AFP, and HBV DNA was noted between those with significant fibrosis and those with mild fibrosis (p>0.05).

Despite the fact that the present study was a prospective study to predict fibrosis in CHB, the limitations of this study include the absence of stage 5 and 6 fibrosis patients and the relatively small sample size.

However, to the best of our knowledge, this is the first study in the literature on fetuin B levels in CHB and HCI patients. Furthermore, there are limited studies in the literature on fetuin A. Fetuin A and B synthesis is known to be significantly reduced in chronic viral hepatitis owing to liver damage. Decreased serum fetuin A and fetuin B levels in CHB and HCI patients shown in the present study may indicate liver injury and impaired liver function, and may consequently be a guide in monitoring disease progression. In the light of these findings, we believe that fetuin A and B levels can be used as noninvasive markers for early detection of high risk of fibrosis in patients with CHB.

**Ethical approval:** The local Institutional Review Board deemed the study exempt from review.

**Informed consent:** Informed consent was obtained from all individuals included in this study.

**Author contributions:** All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

**Competing interests:** Authors state no conflict of interest.

**Research funding:** None declared.

**References**