Pentraxin-3: A Novel Marker for Indicating Liver Fibrosis in Chronic Hepatitis B Patients?

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ABSTRACT

Background: *Pentraxin-3 (PTX-3) is an important marker that plays a role in suppressing inflammation and tissue repair. The aim of this study is to investigate the diagnostic and prognostic characteristics of PTX-3 in chronic hepatitis B (CHB) patients and the relationship between PTX-3 levels and fibrosis.*

Methods: *A total of 52 CHB patients and 40 healthy subjects were included in the study. All of the CHB patients underwent liver biopsy and were then scored using an Ishak histologic scoring system. Blood samples were collected to evaluate the PTX-3 levels.*

Results: *Of the subjects who participated in the study, 53% were female. The PTX-3 levels were determined as 5.63 ng/mL in the control group, and as 0.88 ng/mL in the CHB patient group. PTX-3 levels were found to be 1.19 ng/mL in stage 1, 0.89 ng/mL in stage 2, 0.68 ng/mL in stage 3, and 0.55 ng/mL in stage 4. Of the CHB patients, 44.2% had significant fibrosis, while 55.7% were identified as not having significant fibrosis. The PTX-3 values were 0.64 and 1.0 ng/mL in patients with and without significant fibrosis, respectively. The cut-off value for PTX-3 in predicting the absence of significant fibrosis was estimated as 0.9 ng/mL.*

Conclusion: *The CHB patients were found to have lower serum PTX-3 levels compared to the control group, and these levels decreased even further as the stage of fibrosis progressed. In addition, the significant decrease in PTX-3 levels in patients with stage 1 fibrosis compared to the control group shows that PTX-3 can be used as a non-invasive marker for the early detection of fibrosis ($P < .001$).*

Keywords: *Chronic hepatitis B, fibrosis, Pentraxin-3*

INTRODUCTION

It is estimated that approximately 2 billion people worldwide have been exposed to the Hepatitis B virus (HBV) at some time in their lives, and that about 250 million of them have remained infected with chronic hepatitis B (CHB).[1,2] It is also known that an estimated 1 million people die every year from HBV-related cirrhosis or primary liver cancer.[3] Chronic liver disease occurs as a result of the relation between a progressive wound healing process and inflammatory response.[4] This cycle increases extracellular matrix deposition and leads to liver fibrosis.[5]

Pentraxin-3 (PTX-3) is a recently identified member of the humoral arm of innate immunity, and plays a role in suppressing inflammation and tissue repair.[6,7] PTX-3 is often expressed in different organs by mesenchymal and inflammatory cells following tissue damage, infection, or inflammation.[8] An inverse relationship is observed between PTX-3 levels and inadequate tissue repair, increased collagen deposition, and fibrosis formation in the organs, especially in the liver.[9] This situation has also been associated with defective pericellular fibrinolysis and the defective migration of provisional fibrin-rich inflammatory matrix.[10]

Although progression is slow in hepatitis B patients who do not have liver fibrosis at the time of their first admission, starting an antiviral treatment at the earliest is recommended for patients with significant fibrosis (Ishak score: septal or bridging fibrosis stages 3-6) due to their risk of developing cirrhosis over the years.[11,12] For this reason, assessing the presence of liver fibrosis is of vital importance in determining the prognosis of the disease, the urgency of the treatment, and the potential response to the treatment.[13] In HBV infection, a liver biopsy is the gold standard for evaluating both inflammatory activity and the stage of fibrosis.[14] However, there are some difficulties in practice associated with obtaining liver biopsies, such as the possibility of sampling errors, the invasive nature of the procedure, the high costs it involves, and

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the fact that it requires hospitalization.[15-17] This has led to the search for a new and non-invasive method.

PTX-3 has been defined as a marker in many inflammatory conditions such as atherosclerosis, cancer, and diseases of the respiratory and central nervous systems.[18,19] However, there have been no studies on CHB conducted in this regard. The aim of this study is to investigate the diagnostic and prognostic characteristics of PTX-3 in CHB patients and the relationship between PTX-3 levels and fibrosis.

MATERIALS AND METHODS

This prospective study was approved by the Institutional Review Board of the University on July 19, 2018 and was carried out in accordance with the principles of the Declaration of Helsinki. The participants were informed about the study, and their informed consents for the study were obtained before the collection of clinical information and blood samples.

Patients and Controls

A total of 52 CHB patients who had been admitted to the hospital between August 2018 and February 2019 were included in this study. Simultaneously, a healthy control group was created with 40 people of matching age and sex, with no comorbidities or history of liver disease. The diagnosis of CHB was made according to the criteria of the European Association for the Study of the Liver (EASL).[2]

Patients with a co-infection of hepatitis A, hepatitis C, and human immunodeficiency virus; patients with systemic diseases, patients who could not undergo liver biopsy, and patients with positive autoimmune serology were excluded from the study.

Data Collection

All of the CHB patients underwent liver biopsy. All samples were scored using the Ishak histologic scoring system. The CHB patients were divided into 2 groups, as patients with significant fibrosis (stages 3-6) and patients without significant fibrosis (stages 1-2).[20]

As part of the routine patient analysis, the standard blood tests that were performed included tests for the levels of hepatitis B surface antigen (HBsAg), hepatitis B early antigen (HBeAg), hepatitis B early antibody (antiHBe), HBV viral load (HBVDNA), serum alanine aminotransferase (ALT), and aspartate aminotransferase (AST).

Determination of Plasma Pentraxin 3 Levels

During the study, 5 mL samples of venous blood were collected from the patients into EDTA-containing tubes. Within 40 minutes after collection, the obtained blood samples were centrifuged for 10 minutes at 3500 rpm. The plasma samples were collected and stored at –80°C before assay. Serum PTX-3 levels were analyzed using an ELISA kit (Human PTX-3; Catalog number: 201-12-1939, Biological Technology Co., Ltd, Shanghai, China) in accordance with the manufacturer's instructions.

The measuring range of the Human PTX- 3 ELISA kit was 0.08-20 ng/mL, the intra-assay coefficient of variation CV value was <10%, the inter-assay CV value was <12%, and the minimum detection level was 0.051 ng/mL. Test results were expressed in ng/mL.

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) version 22 package software (IBM Corp.; Armonk, NY, USA) was used for data analysis. Visual (histograms, probability plots) and analytical methods (Kolmogorov–Smirnov/Shapiro–Wilk's test) were applied to check the normal distribution of variables. Continuous variables with normal distribution were presented as mean values (±standard deviation), while data without normal distribution were presented as median values (minimum-maximum). Categorical variables were shown as frequencies and percentages. Depending on the normality of data distribution, the Mann–Whitney *U*-test or the Student's *t*-test were used to compare continuous variables. For multiple comparisons, analysis was carried out using the one-way ANOVA or the Kruskal–Wallis test. Pearson's chi-square test and Fisher's exact test were used to compare categorical variables. Spearman's rho correlation test was used to evaluate correlation. The PTX-3 cut-off value for determining the absence of significant fibrosis was determined using the receiver operating characteristic (ROC) analysis. Differences with a *P* value of <.05 were considered significant.

RESULTS

Of the subjects who participated in the study, 49 (53.3%) were female, while 43 (46.7%) were male ($P > .05$). The mean age of the patients was 38.3 ± 11.4. In terms of age and gender, there was no statistically significant difference between the CHB patient group ($n = 52$) and the control group ($n = 40$) ($P > .05$). The demographic data of the 92 participants included in the study are shown in Table 1.

Table 1. Distribution of Demographic Data Between the Patient and Control Groups

	CHB (*n* = 52)	Control (*n* = 40)	*P*
Female/male	26/26	23/17	.532
Age	39 ± 10.9	38.8 ± 12	.622

CHB, chronic hepatitis B.

The PTX-3 levels were determined as 5.63 (4.21-20) ng/mL in the control group, while PTX-3 levels were significantly lower in the CHB patient group ($P < .001$), at 0.88 (0.39-12) ng/mL. Classification of CHB patients according to a histological scoring system revealed that 21% of patients (*n* = 11) had stage 1 fibrosis, while 34% (*n* = 18) had stage 2, 21% (*n* = 11) had stage 3, and 23% (*n* = 12) had stage 4 fibrosis. However, incomplete cirrhosis (stage 5) and cirrhosis (stage 6) were not detected in any of the patients. Among the patients, PTX-3 levels were determined as 1.19 (1-12) ng/mL at stage 1, 0.89 (0.62-8) ng/mL at stage 2, 0.68 (0.40-4.62) ng/mL at stage 3, and 0.55 (0.39-0.94) ng/mL at stage 4. Accordingly, the PTX-3 levels of stage 1 patients were statistically significantly higher than in patients with fibrosis at stages 2, 3, and 4 ($P = .002$, $P = .001$, and $P < .001$, respectively). There was also a significant difference between stages 2 and 3, and between stages 2 and 4 ($P = .048$ and $P < .001$, respectively). However, there was no significant difference between stages 3 and 4 ($P = .169$). On the other hand, in terms of PTX-3 levels, there was a significant difference between the patients in the control group and those with fibrosis stage 1, and also between the patients of the control group the patients with fibrosis at stages 2, 3, and 4 ($P < .001$).

In the study, 19% (*n* = 10) of our CHB patients had elevated liver enzymes. However, since all of our patients have high HBV DNA load, a liver biopsy was performed. The demographic data and laboratory values of patients according to their stage of fibrosis are presented in Table 2.

There was a negative correlation between plasma PTX-3 level and increased fibrosis ($P = .004$, rho = –0.391). At the same time, there was a negative correlation between serum PTX-3 and APRI and FIB-4 score ($P = .045$, rho = –0.450 and $P = .032$, rho = –0.220 respectively). In addition, there was no correlation between the HISTOLOGY ACTIVITY INDEX and PTX-3 ($P > .05$).

There was significant fibrosis (stage 3-4) in 44.2% (*n* = 23) of CHD patients, and no significant fibrosis was seen in 55.7% (*n* = 29) of the patients ($P > .05$). The PTX-3 values were 0.64 (0.39-4.62) and 1.0 (0.62-12) ng/mL in patients with and without significant fibrosis, respectively, and there was a statistically significant difference between them ($P < .001$). In predicting the absence of significant fibrosis, the AUC was estimated using the ROC curve to be 0.863 for PTX-3 ($P < .001$) (Figure 1), while the cut-off value was found to be 0.97 ng/mL (62.1% sensitivity and 91% specificity) according to results of the likelihood ratio test.

The sample size was decided in accordance with a power analysis (significance level of $P < .05$; power 80%).

DISCUSSION

A significant portion of the world's population is exposed to HBV at some stage of their lives.[3] Over the course of the years, HBV-associated liver damage progresses into liver fibrosis.[5] This progression can be rapid or slow, depending on the extent of active liver inflammation and the degree of damage. However, the primary determinant of this condition is the starting stage of the disease. Advanced fibrosis is associated with decreased survival

Table 2. Demographic Data and Laboratory Values of Patients According to Fibrosis Stage

	Stage 1 (*n* = 11)	Stage 2 (*n* = 18)	Stage 3 (*n* = 11)	Stage 4 (*n* = 12)	*P*
Age	40.1 ± 10.9	38.3 ± 8	40.9 ± 16.9	37.3 ± 9	.900
Female/male (n)	6/5	9/9	5/6	6/6	.796
HBVDNA (10^3 IU/mL)	24.2 (3-579)	19.9 (2.2-329)	113 (8.1-1210)	6.4 (1.9-466672)	.112
AST	24 (16-145)	25 (21-85)	26.2 ± 8.5	21 (17-57)	.500
ALT	30 (20-83)	24 (17-83)	25.9 ± 11.2	18 (11-73)	.434
PLT ($\times 10^3$/μL)	248 ± 69.8	209 (168-304)	202 ± 23.4	231 ± 21.8	.169
PTX-3	1.19 (1-12)	0.89 (0.62-8)	0.68(0.40-4.62)	0.61 ± 0.16	<0.001

AST, aspartate aminotransferase; ALT, alanine aminotransferase; PLT, platelet; PTX-3, pentraxin-3.

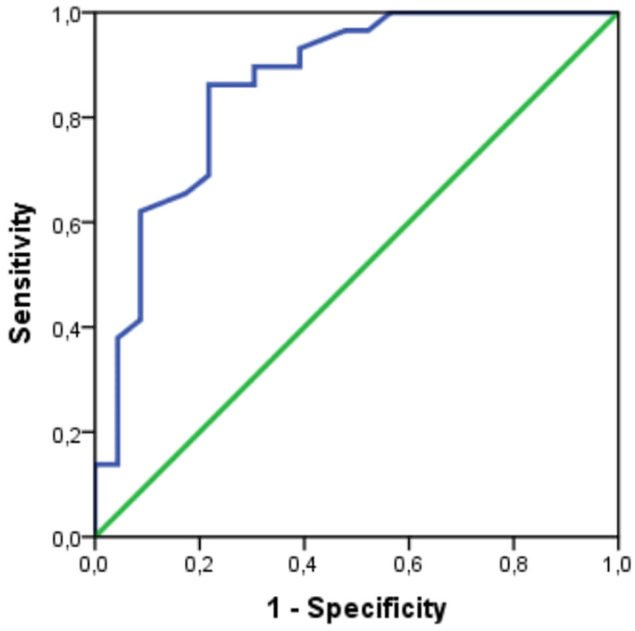

Figure 1. Receiver operating characteristic curves for pentraxin (PTX-3).

and an increased incidence of hepatocellular carcinomo (HCC).[13,21] In this study, we investigated the diagnostic and prognostic characteristics of PTX-3 in CHB patients, and the relationship between PTX-3 levels and fibrosis.

The study of Doni et al.[22] reports that the accumulation of fibrin increases with decreased PTX-3 levels. Another study concluded that interstitial fibrosis-induced acute renal damage can be suppressed through the use of recombinant PTX-3.[23] In the study by Verna et al.,[24] PTX-2 levels were found to be significantly lower in patients with non-alcoholic fatty liver disease (NAFLD) compared to the non-NAFLD control group, and even lower in patients with advanced fibrosis. Serum PTX-2 levels tend to decrease in patients with chronic fibrosis, such as pulmonary fibrosis.[25] The results of our study support this general finding, and the PTX-3 levels of our CHB patients were found to be significantly lower compared to the control group. Furthermore, it was also determined in our patients that there was a significant decrease in serum PTX-3 levels as liver fibrosis increased.

A recently conducted study demonstrated that antiviral treatment leads to a decrease in necro-inflammatory activity and fibrosis.[26] The application of non-invasive methods instead of performing repeated liver biopsies appears to be a far more practical approach for the evaluation of treatment efficacy.[27] This has led to the investigation of non-invasive tests in recent years, with a view to reducing the need for liver biopsy.[28] According to the aspartate aminotransferase (AST)/platelet ratio index (APRI) score suggested by Wai et al.[11] for determining cirrhosis and significant fibrosis in chronic hepatitis C (CHC), the cut-off value has been determined as >2 for cirrhosis and >1.5 for significant fibrosis. The APRI score for predicting the absence of significant fibrosis was found to be <0.5. In their study, Ohta et al.[29] have developed the Fibrosis Index (FI) for predicting fibrosis in CHC patients. Accordingly, a finding of FI <2.1 is considered supportive/indicative of the absence of significant fibrosis. In our study, we evaluated the relationship between PTX-3 and the presence or absence of significant fibrosis. Accordingly, the serum PTX-3 levels were found to be significantly lower in patients with significant fibrosis compared to patients without significant fibrosis. In our study, the cut-off value of PTX-3 was determined to be 0.97 ng/mL in the patient group without significant fibrosis. The results of the present study reveal that significant fibrosis becomes more unlikely with increased levels of PTX-3.

This study demonstrated the performance of plasma PTX-3 in CHB patients for the first time. However, the study does have some limitations. The study had a relatively small sample size, and there were no patients with advanced fibrosis (stage 5 and stage 6 fibrosis) and HCC. As such, our findings need to be verified through studies conducted in multiple centers with larger cohorts.

In conclusion, the serum PTX-3 levels in this study were significantly lower in CHB patients compared to healthy controls, and the PTX-3 levels decreased further as the fibrosis stage progressed. This revealed that PTX-3 is a marker for indicating liver fibrosis. Furthermore, the significant reduction in PTX-3 levels, even in patients with stage 1 fibrosis compared to the control group, shows that PTX-3 is a potential non-invasive determinant that can play a role in the early detection of fibrosis. It is known that HBV-associated liver damage leads to liver fibrosis. For this reason, it is very important to detect fibrosis at an early stage, in order to apply the appropriate treatments. In the light of all this data, it can be said that CHB patients with fibrosis represent an ideal population for studying serum PTX-3 levels.

Ethics Committee Approval: The study was carried out with the approval of Fırat University Faculty of Medicine Ethics Committee (approval number: 20, date: 19.07.2018)..

Informed Consent: Informed consent form was signed by the patients.

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