Original Article

Relationship of serum hepatitis B Virus DNA levels with Hepcidin, Pentraxin-3, Zonulin, and Copeptin in chronic hepatitis B patients

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Abstract

Introduction: This study aims to investigate the serum levels of hepcidin, pentraxin-3 (PTX-3), Zonulin, and Copeptin biomarkers, which can be detected by the more cost-effective and practical ELISA method compared to the quantitatively determined HBV DNA by molecular methods, and to contribute to studies on biomarkers that will facilitate the antiviral treatment management of chronic Hepatitis B patients.

Methodology: Serum samples from 105 patients with HBsAg detected for more than 6 months and 35 control group individuals without any liver or chronic disease were included in the study. The serum HBV DNA levels of the patients were determined by the Real Time PCR (Anatolia, Turkey) method. Patients were divided into three different groups based on their HBV DNA levels (IU/mL): 35 patients with levels between 10^0-10^2, 35 patients with levels between 10^3-10^5, and 35 patients with levels between 10^6-10^8. These groups were evaluated as having mild, moderate, and severe viral loads.

Results: A statistically significant difference was found in serum hepcidin levels between the control group and the group with severe viral load ($p = 0.01$). The difference in serum PTX-3 levels between the control group and the other three groups was statistically significant ($p < 0.001$). A statistically significant difference in Copeptin levels was determined between the group with severe viral load and moderate, mild, control groups ($p = 0.007$; $p = 0.013$; $p = 0.036$).

Conclusions: Important clues have been obtained about markers that can give an idea about HBV DNA, but studies on larger populations are needed.

Key words: HBV DNA; Hepcidin; Pentraxin-3; Zonulin; Copeptin.

Introduction

Hepatitis B virus (HBV) is a major public health problem and one of the most important life-threatening infections in Turkey and worldwide. Countries are classified as high ($\geq$ 8%), intermediate (2-7%), and low (< 2%) prevalence countries based on the percentage of hepatitis B surface antigen (HBsAg) positivity [1]. Various studies conducted in Turkey have reported HBsAg positivity rates between 0.8-5.7%, placing Turkey in the intermediate endemic region. Regionally, the percentage of HBsAg positivity is higher in Southeastern Anatolia and Eastern Anatolia [2,3]. HBsAg positivity lasting more than 6 months suggests a diagnosis of chronic hepatitis B (CHB). Chronic HBV infection can lead to serious complications such as cirrhosis and hepatocellular carcinoma (HCC), which can be fatal.

HBV DNA levels provide information on viral load, disease prognosis, infectivity, and response to treatment in chronic HBV patients [4]. HBV DNA is measured quantitatively by real-time polymerase chain reaction (PCR). Serum HBV DNA levels are used to determine the replication status of chronic HBV infection [5]. Hepcidin is an inflammatory marker involved in iron metabolism. As hepcidin is mainly produced by hepatocytes, pathological changes in the liver affect hepcidin expression. Studies have observed abnormal hepcidin expression in patients with cirrhosis, alcoholic liver disease, chronic hepatitis B, and hepatitis C [6]. PTX-3 is an acute phase reactant similar in structure and function to CRP. PTX-3 activates all three pathways of the complement system: classical, alternative, and lectin. Unlike CRP, PTX-3 can be released by many different cells, including macrophages, endothelial cells, fibroblasts, and synovial cells, in addition to the liver. Alterations in PTX-3 expression have been found in liver disease [7,8].

Zonulin is a protein that is more highly expressed in the intestinal epithelium and has also been shown to be synthesized in brain and heart tissue. It is a signaling protein that regulates intestinal permeability and is released from the epithelial lamina propria. Differences

in Zonulin expression have been found in neoplasia, autoimmune diseases, infectious diseases, and gastrointestinal diseases [9,10].

Arginine vasopressin (AVP), also known as antidiuretic hormone (ADH), is one of the major hypothalamic stress hormones. ADH is a nanopeptide hormone released by the hypothalamus as a result of changes in serum osmolality or hypovolemia and regulates osmotic hemostasis [11]. Copeptin is located in the C-terminal portion of ADH. It is a glycosylated peptide of 39 amino acids, the majority of which is leucine. Copeptin is more commonly used in practice than AVP because it reflects the body's own AVP levels and, unlike AVP, can remain stable in the blood for several days. Elevated Copeptin levels have been found in several cardiac, renal, and infectious diseases [12,13].

New biomarkers are needed for early diagnosis, treatment monitoring, and prognosis of chronic diseases. HBV DNA level, which is the gold standard for viral load, prognosis, and treatment follow-up in chronic HBV patients, is determined by real-time PCR, a molecular technique.

In our study, we aimed to investigate the serum levels of the biomarkers hepcidin, PTX-3, Zonulin, and Copeptin, which can be detected by enzyme-linked immunosorbent assay (ELISA). This method is less expensive and more convenient than the molecular methods used to quantitatively detect HBV DNA. Our aim was to facilitate the management of treatment follow-up in chronic HBV patients and to investigate the correlation between serum HBV DNA levels and the biomarkers investigated.

Methodology

This study was evaluated by the Ethics Committee of Necmettin Erbakan University Pharmaceutical and Non-Medical Device Research Ethics Committee on March 17, 2023, and approved by the decision numbered 2023/4243. This study, project number 23TU18006, was supported by Necmettin Erbakan University Scientific Research Project Coordinatorship.

Between March and November 2023, 105 patients diagnosed with chronic hepatitis B, who had HBsAg detected in their serum for more than 6 months and did not have hepatitis D or hepatitis C co-infection were included in the study. The patient group consisted of individuals without fibrosis or cirrhosis findings on ultrasound (USG) and any other liver diseases and acute or chronic conditions (Table 1). The HBV DNA levels of the patients were determined using the real-time PCR method (Anatolia, Turkey). Results were expressed as International Units per milliliter (IU/mL).

Patients were grouped based on a review of literature, considering viral loads that indicate the need for biopsy and viral loads associated with significant increases in mortality. Patients were divided into three groups: 35 patients with HBV DNA levels between 10^0-10^2 IU/mL, 35 patients with levels between 10^3-10^5 IU/mL, and 35 patients with levels between 10^6-10^8 IU/mL. The groups were evaluated as having mild, moderate, and severe viral loads, respectively. Thirty-five healthy individuals with HBsAg (-) and Anti-HCV (-) as hepatitis markers, without any liver disease such as Wilson's disease, hemochromatosis, alcoholic/toxic hepatitis, autoimmune hepatitis, or elevated ALT/AST enzymes, with normal liver USG findings, no previous HBV treatment, no chronic diseases, and normal hemogram and biochemistry parameters were included as the control group. After the HBV DNA measurement of the patients included in the study, serum samples were stored at -80 °C until the laboratory study in which PTX-3, hepcidin, Zonulin, and Copeptin levels were quantitatively determined by the ELISA method.

In the study, hepcidin, PTX-3, Zonulin, and Copeptin concentrations were obtained by ELISA from serum samples taken from patients or the control group. Hepcidin, PTX-3, and Zonulin levels were determined by the sandwich ELISA method, while Copeptin levels were determined by the competitive ELISA method. Hepcidin, PTX-3, Zonulin, and Copeptin levels were measured using quantitative kits (Elabscience, China) according to the manufacturer's instructions. Results were expressed as nanograms per milliliter (ng/ml) for hepcidin, PTX-3, and Zonulin, and picograms per milliliter (pg/mL) for Copeptin. In our study, hepcidin, PTX-3, Zonulin, and Copeptin levels were compared between the control and patient groups. The relationships of hepcidin, PTX-3, Zonulin, and Copeptin levels with age, gender, and HBV DNA, as well as the correlations between the biomarkers, were analyzed.

Table 1. Inclusion criteria for the study.

Inclusion criteria for the study	Exclusion criteria for the study
Patients diagnosed with Chronic Hepatitis B who have HBsAg in their serum for more than 6 months	Co-infection with HDV, HCV and HIV
	Fibrosis or cirrhosis in USG findings
	Presence of any liver disease such as alcoholic toxic hepatitis, Wilson
	Presence of Chronic Diseases such as Hypertension and Diabetes Mellitus

Table 2. Demographic data and biomarkers levels of control and patient groups.

Variable	Control (n = 35)	Mild (n = 35)	Moderate (n = 35)	Severe (n = 35)	*p*
Age	42.8 (10.6) *	42.8 (13.1) *	43.6 (16.2) *	42.8 (16.8) *	**> 0.99**
Gender					
Male	18 (51%)	18 (51%)	18 (51%)	18 (51%)	**> 0.99**
Female	17 (49%)	17 (49%)	17 (49%)	17 (49%)	
Hepcidin levels	470.4** (257.5-838.0)	968.7** (398.0-1565.0)	962.6** (253.7-1507.5)	979.9** (577.3-1783.0)	
PTX-3 levels	19.4 (12.1) *	46.6 (21.5) *	49.5 (36.9) *	57.3 (39.0) *	
Zonulin levels	49.7** (40.9-60.1)	54.0** (38.4-87.1)	63.3** (47.9-110.6)	65.5** (36.2-91.5)	
Copeptin levels	26.7** (18.9-42.5)	28.9** (18.2-40.0)	34.9** (25.2-44.0)	39.8** (24.2-61.8)	

*Mean (Standard Deviation); **Median value (Q1-Q3).

Statistical Analysis

A total of four groups of 35 participants each, including one control group, and 140 participants were included in the study. According to the G-power analysis performed at the beginning of our study, the power of the study was found to be 0.90264. Numerical variables were expressed as mean and standard deviation or median (Q1-Q3) descriptive statistics. Frequency and percentage statistics were provided for categorical variables. Chi-square and Fisher exact tests were used to analyze categorical variables. Mixed effect models were employed in the analysis of numerical variables. Spearman correlations were calculated between numerical variables. When evaluated by age, the average age of the adults included in the study was considered, and statistical analysis was performed for those under 45 years and those 45 years and older. Analyses were conducted using the R 4.3.1 program (R Core Team, 2023). A $p < 0.05$ was considered significant. The diagnostic decision-making properties of pentraxin-3, hepcidin, Copeptin, and Zonulin levels in predicting hepatitis B disease were assessed by receiver operating characteristics (ROC) curve analysis. In the presence of significant cut-off values, the sensitivity and specificity of these cut-off values were calculated. In evaluating the area under the curve, "1.00-0.90" was considered excellent, "0.90-0.80" was considered good, "0.80-0.70" was considered fair, "0.70-0.60" was considered poor, "0.60-0.50" was considered fair, and a type 1 error level of less than 5% was considered statistically significant.

Results

There was no statistically significant difference in age distribution between the patient groups with mild, moderate, and severe viral loads and the control group ($p > 0.99$, Table 2). Each of the mild, moderate, and severe viral load patient groups, as well as the control group, consisted of 35 individuals. In all groups, 51% (n = 18) were male and 49% (n = 17) were female, and there was no statistically significant difference in gender distribution among the four groups ($p > 0.99$, Table 2).

When serum hepcidin levels were compared statistically in terms of age and gender, higher serum hepcidin levels were found to be statistically significant in adults over 45 years of age and in males ($p < 0.001$; $p = 0.001$, Table 3). Quantitatively measured serum hepcidin levels of the control and patient groups are shown in Table 2.

Statistically, all groups were compared with each other to determine the differences in serum hepcidin levels. A statistically significant difference was found between the control group and the group with a heavy viral load ($p = 0.01$, Table 4).

Table 3. Comparison of biomarker levels according to age and gender.

Variable	Under 45 years old	Over 45 years old	Male	Female	Comparison *p* value of age	Comparison *p* value of gender
Hepcidin	496.4** (214.5-1324.8)	1070.9** (609.4-1957.6)	1248.5** (745.9-1814.5)	398.0** (207.0-779.7)	**< 0.001**	**0.001**
PTX-3	38.5 (27.7) *	49.8 (37.6) *	46.2 (35.3) *	40.0 (28.9) *	**0.133**	**0.538**
Zonulin	61.6** (46.0-99.6)	46.4** (35.3-79.6)	42.9** (27.7-56.5)	80.1** (57.5-116.5)	**0.010**	**< 0.001**
Copeptin	36.3** (25.2-50.5)	27.0** (16.6-44.6)	27.3** (19.8-37.9)	38.3** (26.0-56.7)	**0.017**	**0.191**

*Mean (Standard Deviation); **Median value (Q1-Q3).

Table 4. Comparison of serum hepcidin levels between control and patient groups.

Compared Groups	Estimate	SE	df	t ratio	*p*
Control-Mild	-340.7	235.320	134.0	-1.4	0.472
Control-Moderate	-524.9	235.369	134.0	-2.2	0.120
Control-Severe	-748.5	235.320	134.0	-3.1	**0.010**
Mild-Moderate	-184.1	235.362	134.0	-0.7	0.862
Mild-Severe	-407.8	235.320	134.0	-1.7	0.311
Moderate-Severe	-223.6	235.369	134.0	-0.9	0.778

Table 5. Comparison of serum PTX-3 levels between control and patient groups.

Compared Groups	Estimate	SE	df	t ratio	*p*
Control-Mild	-27.1	6.841	134.0	-3.9	**0.001**
Control-Moderate	-29.3	6.843	134.0	-4.3	**< 0.001**
Control-Severe	-37.8	6.841	134.0	-5.5	**< 0.001**
Mild-Moderate	-2.4	6.843	134.0	-0.3	0.984
Mild-Severe	-10.7	6.841	134.0	-1.5	0.402
Moderate-Severe	-8.2	6.843	134.0	-1.2	0.625

Table 6. Comparison of serum zonulin levels in control and patient groups.

Compared Groups	Estimate	SE	df	t ratio	*p*
Control-Mild	-6.2	15.083	134.0	-0.4	0.976
Control-Moderate	-11.9	15.086	134.0	-0.7	0.858
Control-Severe	-19.2	15.083	134.0	-1.2	0.581
Mild-Moderate	-5.7	15.086	134.0	-0.3	0.981
Mild-Severe	-13.0	15.083	134.0	-0.8	0.824
Moderate-Severe	-7.2	15.086	134.0	-0.4	0.963

When serum PTX-3 levels were analyzed statistically in terms of age and gender, no statistically significant difference was found between age ($p = 0.133$) and gender ($p = 0.538$) and PTX-3 levels. Quantitatively measured serum PTX-3 levels of the control and patient groups are shown in Table 2.

To statistically identify the differences in serum PTX-3 levels among the groups, all groups were compared with each other. A statistically significant difference was found between the control group and the groups with mild, moderate, and severe viral loads ($p = 0.001$; $p < 0.001$; $p < 0.001$, Table 5).

According to the data statistically analyzed for serum Zonulin levels in terms of age and gender, higher serum Zonulin levels were found to be statistically significant in individuals over 45 years of age and in females ($p = 0.01$; $p < 0.001$, Table 3). Quantitatively measured serum Zonulin levels of the control and patient groups are shown in Table 2.

To statistically identify the differences in serum Zonulin levels among the groups, all groups were compared with each other. No statistically significant difference was found among the groups ($p > 0.05$, Table 6).

When serum Copeptin levels were statistically evaluated in terms of age and gender, higher Copeptin levels were found to be statistically significant in individuals over 45 years of age, while no significant difference was found between genders ($p = 0.017$; $p = 0.191$, Table 3). Quantitatively measured serum Copeptin levels of the control and patient groups are shown in Table 2.

To statistically identify the differences in serum Copeptin levels among the groups, all groups were compared with each other. The lower Copeptin levels in the group with a heavy viral load were found to be statistically significant compared to the control group, and the groups with mild and moderate viral loads ($p = 0.007$; $p = 0.013$; $p = 0.036$, Table 7). A positive correlation was detected between hepcidin and PTX-3 and viral load, and a negative correlation was detected between Copeptin and viral load. The correlation of serum hepcidin, PTX-3, Zonulin, and Copeptin markers measured quantitatively by the ELISA method with viral load, and age was examined (Table 8).

The diagnostic decision-making properties of Pentraxin-3 levels in predicting hepatitis B disease were examined by ROC curve analysis (Figure 1). As a result of the ROC analysis, Pentraxin-3 levels had a good

**Table 8.** Correlation analysis (r values) of biomarker levels with age and viral load.

Variable	Age	Viral Load
Hepcidin	0.328**	0.225**
PTX-3	0.195*	0.449**
Zonulin	-0.243**	0.135
Copeptin	0.241**	-0.226**

Table 7. Comparison of serum copeptin levels between control and patient groups.

Compared Groups	Estimate	SE	df	t. ratio	*p*
Control-Mild	-2.7	13.923	134.0	-0.1	0.997
Control-Moderate	-7.6	13.925	134.0	-0.5	0.947
Control-Severe	-45.6	13.922	134.0	-3.7	**0.007**
Mild-Moderate	-4.9	13.925	134.0	-0.3	0.985
Mild-Severe	-42.9	13.923	134.0	-3.0	**0.013**
Moderate-Severe	-38.0	13.925	134.0	-2.7	**0.036**

*Correlation is significant at the 0.05 level; **Correlation is significant at the 0.01 level.

Figure 1. Association between Hepatitis B disease and PTX-3.
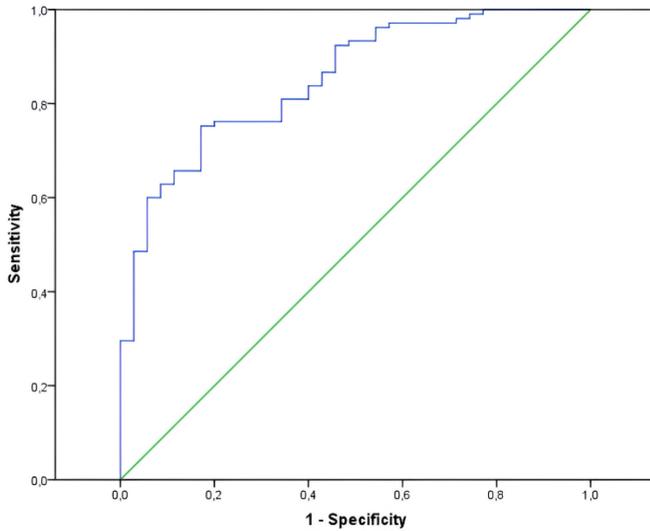
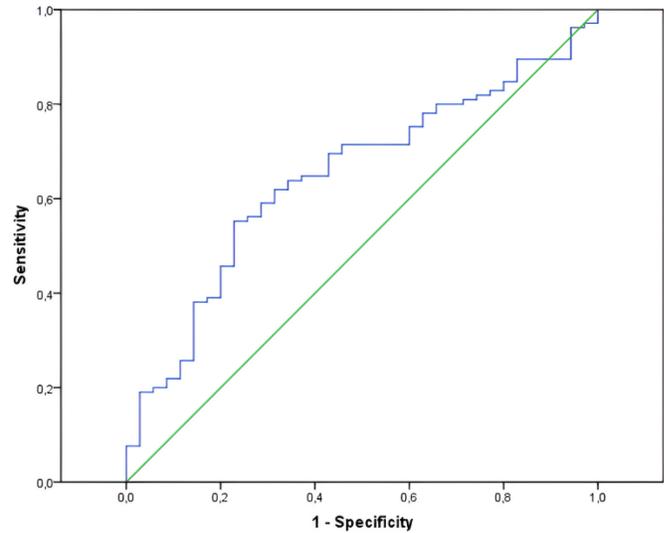**Figure 2.** Relationship between Hepatitis B disease and Hepcidin.

level of statistically significant diagnostic value in predicting hepatitis B disease in patients ($p < 0.001$, AUC = 0.851, 95% CI = 0.783-0.919). The recommended cut-off values for Pentraxin-3 are presented in Table 9. The optimal cut-off value, where the sum of the sensitivity and specificity values reached the highest value, was determined to be 27.62, giving a sensitivity of 0.752 and a specificity of 0.828. The diagnostic decision-making properties of hepcidin levels in predicting hepatitis B disease were examined by ROC curve analysis (Figure 2). As a result of the ROC analysis, hepcidin levels had a low level of statistically significant diagnostic value in predicting hepatitis B disease in patients ($p = 0.009$, AUC = 0.647, 95% CI = 0.548-0.746). The recommended cut-off values for this value are presented in Table 10. The optimal cut-off value, where the sum of the sensitivity and specificity values reached the highest value, was determined to be 883.68, giving a sensitivity of 0.552 and a specificity of 0.771. As a result of the evaluation made by ROC analysis, it was seen that Copeptin ($p = 0.508$; AUC = 0.537) and Zonulin ($p = 0.130$; AUC = 0.586) had no diagnostic value in predicting hepatitis B disease.

Discussion

There is a paucity of studies in the literature to facilitate the diagnosis, treatment and prognostic follow-up of patients with CHB and to reduce costs. Our study investigated the relationship between HBV DNA and various biomarkers to provide information on HBV DNA levels.

A total of 2,998 individuals (1445 males and 1553 females) were included in a multicentre study to investigate the distribution of serum hepcidin levels by age and gender. It was found that hepcidin levels remained constant with age in men, whereas they increased with age in women [14]. In addition, the presence of higher hepcidin levels in men compared to women was found to be statistically significant. Similarly, a study conducted in Bulgaria found that serum hepcidin levels were significantly higher in men than in women [15]. In our study, consistent with the literature, it was statistically significant that serum hepcidin levels were higher in men than in women, were elevated in individuals over the age of 45, and showed a positive correlation with increasing age.

In a study conducted in China, patient and control groups were divided into three categories based on HBV DNA levels: mild, moderate**,** and severe. When

Table 9. Sensitivity, Specificity, and AUC values of Pentraxin-3 for Hepatitis B disease.

Pentraxin-3	Sensitivity	Specificity
10.29	1.000	0.228
16.70	0.952	0.457
19.57	0.905	0.542
27.62	0.752	0.828
33.45	0.629	0.914
35.00	0.600	0.942
58.34	0.295	1.000

$p < 0.001$, AUC = 0.851, 95% CI = 0.783-0.919.

Table 10. Sensitivity, Specificity, and AUC values of Hepcidin for Hepatitis B disease.

Hepcidin	Sensitivity	Specificity
102.91	0.971	0.028
116.35	0.952	0.057
162.01	0.905	0.057
883.68	0.552	0.771
1769.96	0.219	0.914
1820.73	0.200	0.942
2677.53	0.076	1.000

$p = 0.009$, AUC = 0.647, 95% CI = 0.548-0.746.

serum hepcidin levels were analyzed, it was found that the mean serum hepcidin level was significantly higher in the patient group ($p < 0.001$). The same study found a positive correlation between serum hepcidin and HBV DNA levels ($r = 0.383$, $p = 0.001$) [6].

In another study by Wang *et al.*, patients were divided into three groups, diagnosed with CHB, cirrhosis, and HCC. The patient groups had higher mean serum hepcidin levels compared to the control group, with a positive correlation between serum HBV DNA levels and hepcidin levels ($r = 0.48$, $p < 0.01$) [16].

In addition, another study found that serum hepcidin levels were higher in CHB patients than in controls [17]. In a study of cirrhotic patients by Lin *et al.*, serum hepcidin levels were significantly lower in cirrhotic patients compared to controls ($p < 0.001$) [18]. Another study comparing cirrhotic patients and controls also found that serum hepcidin levels were significantly lower in cirrhotic patients ($p < 0.005$) [19].

In our study, similar to the literature data, although the mean values of serum hepcidin levels were higher in the CHB patient groups compared to the control group, a statistically significant difference was found only between the control and severe groups.

In studies in the literature, the reason for lower serum hepcidin levels in cirrhotic patients compared to the control group and higher serum hepcidin levels in patients diagnosed with chronic hepatitis B (CHB) compared to the control group is attributed to liver cell damage in cirrhotic patients.

In addition, our study found a positive correlation between serum HBV DNA levels and serum hepcidin levels, which is consistent with the literature, and a study conducted in China found no statistically significant differences between PTX-3 levels and variables such as age and gender [20]. Similarly, Feder *et al.* reported no significant associations between PTX-3 levels and age or sex [21]. Consistent with these findings, our study also found no statistically significant differences in PTX-3 levels when analyzed by age and gender.

In a study including three groups of patients diagnosed with CHB, cirrhosis, and hepatocellular carcinoma (HCC) and a control group, the elevated PTX-3 levels in the patient groups were found to be statistically significant compared to the control group ($p < 0.001$) [22]. In a study conducted in Turkey, it was statistically significant that as the stage of liver fibrosis due to CHB increased, PTX-3 levels decreased ($p < 0.01$) [23]. Furthermore, in HCC patients, the increase

in serum PTX-3 levels was found to be statistically significant in relation to decreased survival time [20].

In our study, similar to the literature, a statistically significant difference was found between the control group and the mild, moderate, and severe groups. Although previous studies have reported higher PTX-3 levels in cirrhotic patients, we believe that the observed decrease in PTX-3 levels as the stage of cirrhosis progresses may be related to the viral load of the patients included in the study. To the best of our knowledge, our study is the first in the literature to directly examine the relationship between HBV DNA levels and PTX-3 levels, thereby investigating the association between viral load and PTX-3 in hepatitis B patients. We believe this is a significant advantage of our study over others. Consistent with the literature, our study also found a positive correlation between serum PTX-3 and HBV DNA levels, suggesting an association between PTX-3 and viral load.

In a study by Sapone *et al.*, no correlation was found between serum Zonulin and age or sex [24]. Similarly, another study conducted in Poland found no significant difference between serum Zonulin levels and gender or age [25]. In our study, it was significant that serum Zonulin levels decreased with increasing age, were higher in women than in men, and showed a negative correlation with age.

In a study by Pietrukaniec *et al.* on a group of patients diagnosed with cirrhosis, Zonulin levels were found to be significantly lower in cirrhotic patients than in controls ($p < 0.001$) [26]. Conversely, another study of cirrhotic patients reported higher serum Zonulin levels in the patient group compared to the control group, although this difference was not statistically significant [26]. In a study conducted in Turkey, in which patients diagnosed with CHB were divided into three groups (mild, moderate, and severe) based on viral load, serum Zonulin levels were found to be statistically significantly lower in CHB patients than in the control group ($p < 0.001$). A significant difference in Zonulin levels was only observed between the mild and severe viral load groups. In addition, a negative correlation ($r = -0.510$, $p < 0.05$) was found between serum HBV DNA levels and Zonulin levels [10].

In our study, no statistically significant difference was found between the control and patient groups, nor between the patient groups themselves. No correlation was found between serum HBV DNA levels and Zonulin levels. To our knowledge, our study is the second in the literature to directly investigate the relationship between serum HBV DNA levels and Zonulin levels. There is no consensus in the literature

on the relationship between serum Zonulin levels in patient groups diagnosed with cirrhosis and CHB.

In a study conducted in Turkey examining serum Copeptin levels in relation to age and gender, no significant difference was found between age, gender, and Copeptin levels [27]. In another study investigating Copeptin levels, no association was found between age and Copeptin levels [28]. In our study, no significant difference was found between Copeptin levels and gender, while a significant difference was found between Copeptin levels and age, and a positive correlation was found between age and Copeptin levels. In a study conducted in Egypt, a statistically significant increase in serum Copeptin levels was found in patients diagnosed with cirrhosis who developed complications compared to those who did not develop complications [29]. In a study conducted in Turkey, in which patients diagnosed with CHB were divided into three groups (mild, moderate, and severe) based on viral load, the decrease in Copeptin levels was statistically significant in the patient group compared to the control group ($p < 0.001$). No statistically significant difference in Copeptin levels was found between the mild, moderate, and severe groups ($p > 0.05$). The same study found a negative correlation (r = -0.415, $p = 0.023$) between serum HBV DNA levels and serum Copeptin levels [10].

In our study, a statistically significant difference was found between the low Copeptin levels in the severe group and the Copeptin levels in the control group, as well as in the mild and moderate viral load groups. Similar to the study by Çalgın *et al.*, our study also found a negative correlation between serum HBV DNA levels and Copeptin levels.

To our knowledge, there is only one study in the literature that examined the relationship between Zonulin and Copeptin and HBV DNA with a very small patient population, and our study is the first study to directly examine the relationship between PTX-3 and HBV DNA. The specificity of our study stems from the fact that it is the first study to examine the relationship between hepcidin, PTX-3, Zonulin, and Copeptin levels and HBV DNA together. In this study, a positive correlation was found between hepcidin, PTX-3 levels, and HBV DNA, and a negative correlation was found between Copeptin and HBV DNA levels in chronic hepatitis B patients. In the present study, PTX-3 values were found to have a statistically significant diagnostic value in predicting Chronic Hepatitis B disease in patients. Hepcidin was found to have a weak statistically significant diagnostic value in predicting Chronic Hepatitis B disease. Zonulin and Copeptin were found to have no diagnostic significant value in Chronic Hepatitis B patients.

Conclusions

In this study, important insights have been obtained regarding various promising biomarkers that can be detected through more practical and cost-effective ELISA tests, which could facilitate the follow-up process of patients diagnosed with CHB and provide information about HBV DNA. Studies involving larger patient populations and more detailed categorization of patient groups are crucial for confirming these insights.

Authors' contributions

BE: Writing the article and collecting samples, interpreting data and analysis; MO: Final check of the written article and determination of the topic, interpreting analysis

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Conflict of interests

No conflict of interests is declared.

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