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Serum Beta-Klotho as a damage-related biomarker in chronic hepatitis B: Correlations with non-invasive fibrosis and inflammation indices

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ABSTRACT

Background: Chronic hepatitis B (HBV) infection causes significant morbidity. Liver biopsy, the gold standard for fibrosis assessment, is invasive, highlighting the need for non-invasive biomarkers. β-Klotho, a transmembrane protein, may be released during hepatocyte injury. This study aimed to evaluate serum β-Klotho in treatment-naïve chronic HBV patients and its correlation with inflammation and fibrosis indices.

Methods: Sixty-seven treatment-naïve HBV patients were divided by viral load (<10^5 IU/mL, n=34; ≥10^5 IU/mL, n=33), with 18 healthy controls. Serum β-Klotho was measured via ELISA. Inflammation indices (SII, SIRI, PLR, NLR, LMR, PNR) and non-invasive fibrosis indices (APRI, GAPI, API, PAPAS, others) were calculated. Spearman correlation and ROC analysis were performed.

Results: Median serum β-Klotho levels tended to be higher in patients with increased HBV DNA levels; however, no statistically significant difference or correlation was observed (p=0.052; p=0.813). Positive correlations were found with SIRI (r=0.389, p=0.001) and GAPI (r=0.270, p=0.027), negative with LMR (r=-0.269, p=0.028) and PNR (r=-0.322, p=0.008). ROC analysis showed limited diagnostic value (AUC=0.674, p=0.024), with high specificity (94.4%) but low sensitivity (46.3%). No correlation with fibrosis stage was observed.

Conclusions: Serum β-Klotho tends to increase with viral load, reflecting hepatocellular injury and systemic inflammation rather than established fibrosis. It may serve as a novel non-invasive damage-associated biomarker in chronic HBV, though diagnostic sensitivity is limited.

Keywords: Beta-Klotho, chronic hepatitis B, HBV DNA, liver injury, non-invasive fibrosis and inflammation indices

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INTRODUCTION

Hepatitis B virus (HBV) infection continues to pose a major threat to global health due to its substantial clinical burden and associated deaths [1]. Recent World Health Organization estimates indicate that approximately 254 million individuals worldwide are chronically infected with HBV, and nearly one million people die each year from liver-related complications, including cirrhosis and hepatocellular carcinoma [1,2]. Turkey is located in the intermediate endemic class and various studies conducted in Turkey have reported HBsAg positivity rates between 0.8% and 5.7%, placing Turkey in the intermediate endemic region [3]. The clinical course of chronic HBV infection is influenced by the dynamic balance between viral replication and host immune responses. As a result, patients may exhibit a wide range of outcomes, from clinically silent infection to progressive hepatic fibrosis and end-stage liver disease [4]. This variability complicates risk evaluation and treatment planning, underscoring the need for reliable tools that can accurately represent disease activity.

Accurate staging of liver fibrosis is essential in chronic liver disorders for predicting prognosis and determining management strategies. Although liver biopsy is considered the reference method for evaluating fibrosis severity, its invasive nature, procedural risks, sampling variability, and cost hinder widespread use in clinical practice [5,6]. Therefore, attention has shifted toward non-invasive prognostic markers derived from routine hematological and biochemical tests. Indices such as the aminotransferase-to-platelet ratio index (APRI), γ-glutamyl transferase-to-platelet ratio (GAPI), systemic immune-inflammation index (SII), and platelet-to-lymphocyte ratio (PLR) have been investigated as useful surrogate tools for assessing hepatic inflammation and fibrotic remodeling [7].

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β-Klotho is a membrane-bound protein of 1,043 amino acids that is highly expressed in metabolically active tissues, particularly the liver, adipose tissue, and pancreas [8,9]. It functions as an essential co-receptor for fibroblast growth factors FGF19 and FGF21, enabling receptor binding affinity and triggering downstream signaling pathways [10,11]. Through the FGF–FGFR–β-Klotho axis, this protein plays key roles in metabolic regulation, including bile acid homeostasis and glucose and lipid metabolism [12].

Recent research has highlighted a context-dependent involvement of β-Klotho in liver injury. Pro-inflammatory mediators such as IL-1β and TNF-α have been shown to downregulate β-Klotho gene expression in conditions like alcoholic fatty liver disease, where mRNA levels decline in parallel with worsening fibrosis [13,14]. These observations suggest a protective role against hepatocellular damage. Conversely, higher circulating concentrations of β-Klotho have been reported in advanced liver disease, including HBV-related cirrhosis and hepatocellular carcinoma, despite diminished hepatic expression [15]. This discrepancy supports the hypothesis that β-Klotho might be released into the bloodstream as a consequence of hepatocyte injury and apoptotic processes [16]. To our knowledge, no study has yet comprehensively analyzed the association of serum β-Klotho with systemic inflammation and multiple non-invasive fibrosis indices in untreated chronic HBV infection.

The link between HBV DNA burden and circulating β-Klotho levels remains insufficiently characterized. Considering the potential dissociation between hepatic synthesis and serum detection, there is a growing need for further investigation. In this study, we compared serum β-Klotho concentrations in treatment-naïve chronic HBV patients and healthy individuals, evaluated its ability to discriminate disease using ROC analysis, and examined its relationship with inflammation and fibrosis markers.

METHODS

This study was approved by the Non-Drug and Medical Device Research Ethics Committee of the Faculty of Medicine, Necmettin Erbakan University Hospital on 7 March 2025 (Decision No: 2025/5614). Written informed consent was obtained from all participants prior to enrollment.

Study population

Treatment-naïve individuals with chronic HBV infection (defined as HBsAg positivity for more than 6 months) were recruited from the Medical Microbiology Laboratory of Necmettin Erbakan University Hospital. Serum HBV DNA quantification was performed by real-time PCR (Anatolia, İstanbul, Türkiye). A Nucleic Acid Isolation and PCR Setup Robot was used for nucleic acid isolation from patient serum. The Bosphore HBV Quantification Kit, which is compatible with the device, included a master mix, distilled water for the negative control, the internal control and the positive control, as well as four standard samples. The nucleic acid isolation kit included a viral DNA isolation kit, proteinase K and carrier RNA solutions. First, the patient serum samples were centrifuged and prepared for feeding into the Nucleic Acid Isolation and PCR Setup Robot. Next, the standard samples, carrier RNA and proteinase K samples from the Bosphore HBV Quantification Kit were vortexed. The master mix, negative control, internal control, carrier RNA solutions and proteinase K samples were then placed into the Nucleic Acid Isolation and PCR Setup Robot in accordance with the instructions of the device. Following the completion of the nucleic acid isolation process, the PCR plates were transferred to a Real Time PCR device, and HBV DNA levels were quantitatively measured in IU/mL.

Participants were stratified into two subgroups according to viral load: low viral load ($<10^5$ IU/mL) and High viral load ($\geq 10^5$ IU/mL). Additionally, 18 healthy volunteers negative for both HBsAg and anti-HCV, without a history of liver pathology (e.g., autoimmune hepatitis, hemochromatosis, Wilson's disease, alcoholic or toxic hepatitis), infectious or chronic systemic disorders, and with normal ALT/AST levels were included as the control cohort. Eligibility criteria for the study population were as follows:

Inclusion criteria: Having been diagnosed with chronic hepatitis B with HBsAg positivity for at least 6 months; and not having received antiviral treatment.

Exclusion criteria: Coinfection with hepatitis C (HCV), hepatitis D (HDV). or HIV; Any history of malignancy or current malignant disease; Alcohol consumption >30 g/day for men and >20 g/day for women; Other chronic liver diseases such as autoimmune hepatitis, non-alcoholic fatty liver disease (NAFLD), hemochromatosis and Wilson's disease; Acute infection, febrile illness or systemic inflammation within the last month; Presence of rheumatological or autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel diseases etc; Type 1 or Type 2 diabetes mellitus; or chronic kidney disease (glomerular filtration rate <60 mL/min/1.73 m^2).

Beta-Klotho and inflammatory indices

Serum samples were stored at –80 °C until β-Klotho quantification was performed using a commercial ELISA kit (BT LAB, Shanghai, China; Cat. No: E2782Hu). β-Klotho concentrations were expressed in nmol/L, as provided by the manufacturer's calibration standards (BT LAB, Human Beta-Klotho, ELISA). No unit conversion or mathematical transformation was applied during data analysis. The Systemic Immune-Inflammation Index

(SII) was calculated as Platelet × Neutrophil / Lymphocyte. The Systemic Inflammation Response Index (SIRI) was calculated as Neutrophil × Monocyte/ Lymphocyte. Hematological parameter indices Platelet/ Lymphocyte (PLR), Neutrophil/ Lymphocyte (NLR), Lymphocyte-to-Monocyte ratio (LMR), Platelet-Neutrophil ratio (PNR), MPV/ Platelet, and PDW/ Platelet were investigated.

Non-invasive fibrosis indices and liver biopsy

Additionally, non-invasive fibrosis indices Aspartate aminotransferase (AST)/ Alanine aminotransferase (ALT) ratio (AAR), AST-Platelet ratio Index (APRI), Age-Platelet Index (API), AST/ Platelet / Gammaglutamyl transpeptidase (GGT)/ Alphafetoprotein (AFP) (APGA), Platelet/ Age/ Phosphatase (ALP)/ AFP/ AST (PAPAS), AST-GGT ratio (AGR), GGT-Platelet ratio (GAPI), and AAR/ Platelet score (AARP) were investigated. The β-klotho relationship was examined statistically with these ratios. Fibrosis grading was determined by histopathological assessment via liver biopsy.

Statistical analysis

All statistical analyses were carried out using the Statistical Package for the Social Sciences (SPSS). Categorical variables were presented as counts and percentages, whereas continuous parameters were expressed as median and interquartile ranges (Q1–Q3). The distribution pattern of quantitative data was assessed both visually through histogram inspection and analytically using the Shapiro–Wilk test. For comparisons between two independent groups, the Mann–Whitney U test was applied in cases where normality was not met, while ANOVA or the Kruskal–Wallis test was used to analyze more than two groups depending on distribution status. Associations between non-normally distributed numerical variables were evaluated using Spearman's correlation analysis, with coefficients interpreted as negligible (0.00–0.19), weak (0.20–0.39), moderate (0.40–0.69), strong (0.70–0.89), or very strong (0.90–1.00). The diagnostic performance of β-Klotho in distinguishing HBV-related disease was investigated using receiver operating characteristic (ROC) curve analysis, and significant cut-off values were further assessed by calculating corresponding sensitivity and specificity rates. Area under the curve (AUC) values were classified as excellent (0.90–1.00), good (0.80–0.90), moderate (0.70–0.80), poor (0.60–0.70), or non-informative (0.50–0.60). A p value <0.05 was regarded as statistically significant.

RESULTS

A total of 85 individuals were included in the study, comprising 18 healthy controls, 34 patients with an HBV DNA level $\leq 10^5$ IU/mL, and 33 patients with an HBV DNA level $>10^5$ IU/mL. Overall, the sex distribution was balanced, with 42 males (49.4%) and 43 females (50.6%). The hematological parameters, inflammatory indices, and non-invasive fibrosis indices of all groups are summarized in Table 1. No statistically significant differences were observed between the groups with respect to age, sex.

The median β-Klotho values were 3.880 nmol/L (2.155-7.957) in the control group, 6.232 nmol/L (3.650-15.654) in the group with HBV DNA below 10^5 IU/mL, and 16.376 nmol/L (3.235-22.581) in the group with HBV DNA above 10^5 IU/mL. No statistically significant difference was found between the groups in terms of β-Klotho levels (p=0.052). When β-Klotho concentrations were analyzed according to fibrosis stages, no statistically significant differences were detected between patient subgroups (p=0.350). No statistically significant correlation was found between β-Klotho levels and fibrosis stage (r=0.011, p=0.949). No statistically significant correlation was found between β-Klotho levels and viral load (r=0.029, p=0.813).

A statistically significant difference was found when comparing platelet/ lymphocyte ratios between groups (p=0.010). Post hoc analyses revealed that this difference was due to patients with viral loads greater than 10^5 IU/mL having lower platelet/ lymphocyte ratios than those with viral loads of 10^5 IU/mL and below. A statistically significant difference was found when comparing lymphocyte/ monocyte ratios between groups (p=0.020). Post hoc analyses revealed that this difference was due to the control group having higher lymphocyte/ monocyte ratios than the other groups.

A statistically significant difference was found when comparing MPV/ Platelet values between groups (p<0.001). Post hoc analyses determined that the difference was due to the control group having a lower MPV/ platelet value than the other groups. A statistically significant difference was found when comparing PDW/ Platelet values between groups (p=0.011). Post hoc analyses determined that the difference was due to the control group having a lower PDW/ platelet value than patients with a viral load greater than 10^5 IU/mL. A statistically significant difference was found when comparing SII values between groups (p=0.030). Post hoc analyses determined that the difference was due to the control group having a higher SII value than patients with a viral load greater than 10^5 IU/mL. A statistically significant difference was found when comparing AAR values between groups (p=0.016). Post hoc analyses determined that the difference was due to the control group having a higher AAR value than patients with a viral load greater than 10^5 IU/mL. A statistically significant difference was found when comparing API values between groups (p=0.005). Post hoc analyses determined that the difference was due to the control group having a lower API value than

Table 1. Blood parameters, inflammation, and fibrosis indices of the patient and control groups

	Control		Patient	
	Mean ± SD	**Median [IQR]**	**Mean ± SD**	**Median [IQR]**
Age (years)	45.6 ± 7.3	45.5 [38.0-50.2]	49.7 ± 14.3	48.0 [41.5-59.0]
Hemoglobin (g/dL)	14.45 ± 0.95	14.30 [13.75-15.17]	14.44 ± 6.64	13.80 [12.50-15.70]
WBC (×10³/uL)	7.66 ± 0.45	7.62 [7.25-8.01]	7.49 ± 3.74	7.21 [5.60-8.29]
Platelet (×10³/uL)	321.6 ± 61.7	322.5 [274.7-371.2]	219.6 ± 80.7	229.0 [172.0-272.0]
Neutrophil (×10³/uL)	4.963±1.619	4.880 [3.692-6.510]	4.176 ± 2.047	4.270 [2.830-5.130]
Monocyte (×10³/uL)	0.55 ± 0.25	0.59 [0.30-0.75]	0.79 ± 0.962	0.53 [0.42-0.69]
Lymphocyte (×10³/uL)	2.92 ± 1.13	2.34 [1.65-2.91]	2.35 ± 1.84	2.25 [1.62-2.79]
MPV (fL)	8.51 ± 1.10	8.35 [7.55-9.37]	10.06 ± 1.99	10.30 [9.70-11.00]
PDW (%)	12.9 ± 2.4	12.0 [10.8-15.6]	11.8 ± 2.7	12.2 [10.5-13.4]
ALP (IU/L)	67.2 ± 14.9	62.5 [54.2-82.2]	107.4 ± 56.2	95.0 [68.0-127.0]
CRP (mg/L)	0.82 ± 0.66	0.69 [0.40-0.87]	11.23 ± 20.81	2.75 [0.90-11.28]
GGT (U/L)	35.8 ± 16.9	36.0 [17.7-52.2]	49.2 ± 61.5	24.0 [16.0-53.0]
LDH (U/L)	167.9 ± 22.0	175.5 [148.5-185.2]	246.1 ± 145.8	207.0 [165.0-256.0]
AST (U/L)	23.8 ± 8.1	26.0 [16.8-30.5]	91.3 ± 310.4	28.1 [17.9-57.9]
ALT (U/L)	20.2 ± 8.3	19.0 [13.1-26.2]	130.1 ± 383.5	30.9 [16.7-80.9]
AFP (ng/mL)	1.57 ± 0.99	1.36 [0.84-2.58]	765.76 ± 6153.61	2.14 [1.28-3.91]
Beta-Klotho (nmol/L)	5.72 ± 4.78	3.88 [2.15-7.95]	11.36 ± 11.02	6.39 [3.15-19.15]
PLR	131.5 ± 74.0	115.2 [83.4-155.3]	119.5 ± 93.5	106.8 [80.7-138.8]
NLR	1.96 ± 1.01	1.68 [1.14-2.45]	2.32 ± 2.18	1.79 [1.27-2.46]
LMR	6.62 ± 5.10	4.78 [3.82-7.22]	3.81 ± 1.66	3.83 [2.59-4.84]
PNR	70.22 ± 23.21	62.81 [53.65-76.04]	60.91 ± 28.32	57.90 [44.05-72.91]
MPV/PLT	0.027 ± 0.008	0.026 [0.021-0.033]	0.099 ± 0.362	0.045 [0.036-0.061]
PDW/PLT	0.042 ± 0.014	0.040 [0.031-0.048]	0.117 ± 0.431	0.053 [0.039-0.070]
SII	650.9 ± 387.0	578.7 [384.7-918.4]	500.1 ± 404.2	414.6 [254.1-605.4]
SIRI	0.94 ± 0.44	0.84 [0.64-1.22]	1.92 ± 3.97	1.04 [0.58-1.60]
AAR	1.48 ± 0.95	1.43 [0.69-1.94]	0.94 ± 0.42	0.87 [0.63-1.16]
API	0.146 ± 0.034	0.143 [0.112-0.172]	0.593 ± 1.816	0.201 [0.142-0.363]
APRI	0.077 ± 0.031	0.085 [0.047-0.101]	0.791 ± 2.368	0.126 [0.078-0.328]
AAR/PLT	0.004 ± 0.003	0.004 [0.002-0.006]	0.012 ± 0.048	0.003 [0.002-0.005]
GAPI	0.109 ± 0.045	0.108 [0.065-0.157]	1.014 ± 5.142	0.109 [0.062-0.289]
AGR	0.902 ± 0.635	0.724 [0.429-1.268]	1.931 ± 2.913	1.020 [0.644-1.925]
APGA	0.013 ± 0.045	0.002 [0.000-0.006]	0.006 ± 0.013	0.002 [0.000-0.005]
PAPAS	0.008 ± 0.014	0.004 [0.002-0.007]	0.001 ± 0.002	0.000 [0.000-0.001]

Results are reported either as mean ± standard deviation or as median with [interquartile range]. Abbreviations: AAR – AST-to-ALT ratio, AFP – alpha-fetoprotein, AGR – AST-to-GGT ratio, ALP – alkaline phosphatase, ALT – alanine aminotransferase, AST – aspartate aminotransferase, APGA – AST / platelet count / GGT / AFP, API – age-platelet index, APRI – AST-to-PLT ratio index, CRP – C-reactive protein, GAPI – GGT-to-PLT ratio index, GGT – gamma-glutamyl transferase, LDH – lactate dehydrogenase, LMR – lymphocyte-to-monocyte ratio, MPV – mean platelet volume, NLR – neutrophil-to-lymphocyte ratio, PAPAS – Platelet/ Age/ ALP/ AFP/ AST, PDW – platelet distribution width, PLR – platelet-to-lymphpcyte ratio, PLT – platelet count, PNR – platelet-to-neutrophil ratio, SII – systemic immune-inflammation index, SIRI – systemic inflammation response index, WBC – white blood cell count.

the other groups. A statistically significant difference was found when comparing APRI values between groups (p<0.001). Post hoc analyses determined that the difference was due to the APRI value being higher in those with a viral load greater than 10^5 IU/mL than the other groups. A statistically significant difference was found in GAPI values when compared between groups (p=0.018). Post hoc analyses determined that the difference was due to the fact that patients with a viral load of 10^5 IU/mL or less had lower GAPI values than those with a viral load greater than 10^5 IU/mL. A statistically significant difference was found in AGR values when compared between groups (p=0.044). Post hoc analyses determined that the difference was due to the fact that the control group had lower AGR values than those with a viral load greater than 10^5 IU/mL. A statistically significant difference was found in PAPAS values when compared between groups (p=0.044). Post hoc analyses revealed statistically significant differences among all groups (Table 2).

Correlation analyses demonstrated a statistically significant but weak negative association between serum β-Klotho and the lymphocyte-to-monocyte ratio (r=−0.269, p=0.028), as well as between β-Klotho and the platelet-to-neutrophil ratio (r=−0.322, p=0.008). In contrast, β-Klotho exhibited a weak yet statistically significant positive correlation with the systemic inflammation response index (SIRI) (r=0.389, p=0.001) and the GGT-to-platelet ratio (GAPI) (r=0.270, p=0.027).

There was a statistically significant, moderate negative correlation between fibrosis stage and platelet-lymphocyte ratio (r=-0.585, p<0.001), a statistically significant, low negative correlation between neutrophil/ lymphocyte ratio (r=-0.357, p=0.028), a statistically significant, low positive correlation between MPV and platelet ratio

Table 2. Hematological parameter indices, non-invasive fibrosis indices, age and sex comparison between groups

	Control group [n=18)	Viral load ≤ 10^5 IU/mL [n=34)	Viral load > 10^5 IU/mL [n=33)	*p* value
Male, n [%)	9 (50.0)	17 (50.0)	16 (48.5)	0.991 [1]
Age [years)	45.6 ± 7.3	51.9 ± 12.9	49.7 ± 17.9	0.323 [2]
Beta Klotho [nmol/L)	3.880 [2.155-7.957]	6.232 [3.650-15.654]	16.376 [3.235-22.581]	0.052 [3]
PLR	115.2 [83.4-155.3]	124.3 [89.6-151.6]	90.1 [66.0-115.5]	0.010 [3]
NLR	1.68 [1.14-2.45]	2.00 [1.45-2.64]	1.69 [1.23-2.37]	0.560 [3]
LMR	4.78 [3.82-7.22]	3.79 [2.87-4.70]	3.83 [2.37-5.01]	0.020 [3]
PNR	62.81 [53.65-76.04]	62.20 [47.68-81.55]	52.74 [32.73-71.59]	0.054 [3]
MPV/PLT	0.026 [0.021-0.033]	0.045 [0.033-0.050]	0.045 [0.036-0.071]	< 0.001 [3]
PDW/PLT	0.040 [0.031-0.048]	0.052 [0.040-0.060]	0.054 [0.039-0.087]	0.011 [3]
SII	578.7 [384.7-918.4]	454.4 [320.7-722.9]	380.5 [188.1-532.0]	0.030 [3]
SIRI	0.84 [0.64-1.22]	1.05 [0.58-1.63]	0.91 [0.58-1.74]	0.569 [3]
AAR	1.43 [0.69-1.94]	0.96 [0.74-1.27]	0.75 [0.48-1.06]	0.016 [3]
API	0.143 [0.112-0.172]	0.202 [0.151-0.280]	0.200 [0.123-0.417]	0.005 [3]
APRI	0.085 [0.047-0.101]	0.081 [0.051-0.126]	0.274 [0.125-0.862]	< 0.001 [3]
AAR/PLT	0.004 [0.002-0.006]	0.003 [0.002-0.005]	0.003 [0.002-0.006]	0.722 [3]
GAPI	0.108 [0.065-0.157]	0.082 [0.061-0.141]	0.251 [0.067-0.662]	0.018 [3]
AGR	0.724 [0.429-1.268]	0.898 [0.670-1.354]	1.375 [0.584-2.729]	0.044 [3]
APGA	0.002 [0.000-0.006]	0.002 [0.000-0.003]	0.003 [0.000-0.008]	0.800 [3]
PAPAS	0.004 [0.002-0.007]	0.001 [0.000-0.003]	0.000 [0.000-0.001]	< 0.001 [3]

1 Chi-square test was performed [numbers and column percentages are reported). 2 ANOVA test was performed [mean ± SD is reported). 3 The Kruskal-Wallis test was performed [median with [interquartile range] is reported). Abbreviations: AAR – AST-to-ALT ratio, AGR – AST-to-GGT ratio, APGA – AST / platelet count / GGT / AFP, API – age-platelet index, APRI – AST-to-PLT ratio index, GAPI – GGT-to-PLT ratio index, LMR – lymphocyte-to-monocyte ratio, MPV – mean platelet volume, NLR – neutrophil-to-lymphocyte ratio, PAPAS – Platelet/ Age/ ALP/ AFP/ AST, PDW – platelet distribution width, PLR – platelet-to-lymphpcyte ratio, PLT – platelet count, PNR – platelet-to-neutrophil ratio, SII – systemic immune-inflammation index, SIRI – systemic inflammation response index.

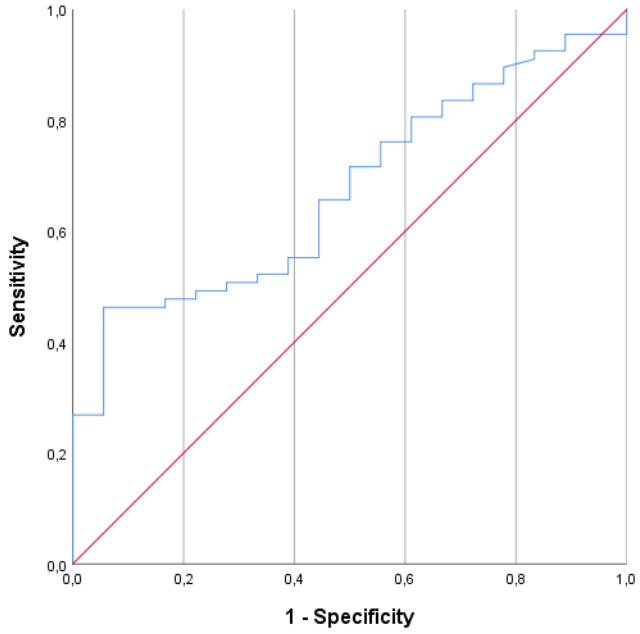

Figure 1. ROC curve assessing the diagnostic accuracy of β-Klotho for chronic HBV infection.

(r=0.391, *p*=0.015), a statistically significant, low positive correlation between PDW and platelet ratio (r=0.399, *p*=0.013), a statistically significant, moderate negative correlation between SII value (r=-0.643, *p*<0.001), a statistically significant, moderate positive correlation between API value (r=0.546, *p*<0.001), a statistically significant, moderate positive correlation between APRI value (r=0.425, *p*=0.008), and a statistically significant, moderate positive correlation between GAPI value (r=0.564, *p*<0.001). A moderately statistically significant negative correlation (r=-0.429, *p*=0.008) was found between PAPAS values and the study results (Table 3).

The receiver operating characteristic (ROC) curve assessing the diagnostic accuracy of β-Klotho for chronic HBV infection is presented in Figure 1. Although its discriminative ability was limited, a statistically significant diagnostic performance was noted (*p*=0.024), with an AUC value of 0.674. The threshold levels generated by ROC analysis are provided in Table 4. The optimal cut-off point was identified as 11.525 nmol/L, yielding a sensitivity of 46.3% and a specificity of 94.4%.

DISCUSSION

This study investigated the relationship between serum β-Klotho levels, viral load, inflammation, and non-invasive fibrosis indices in treatment-naïve patients with chronic hepatitis B virus (HBV) infection. Our findings indicate a gradual increase in serum β-Klotho levels with higher viral load, consistent with previous studies. For example, Miao et al. reported a stepwise increase in serum β-Klotho from healthy individuals to patients with HBV-associated cirrhosis and hepatocellular carcinoma [15].

Conversely, studies examining hepatic tissue have shown that β-Klotho mRNA levels decrease with fibrosis progression, as observed in both viral and alcoholic liver disease [13]. This paradox—decreased hepatic expression but increased serum levels—suggests that β-Klotho is shed from the hepatocyte membrane during ongoing cell damage and apoptosis, rather than reflecting increased hepatic synthesis [16].

Table 3. Correlation of inflammation and non-invasive fibrosis indices with viral load, β-Klotho values, and fibrosis

		Viral Load	Beta-Klotho	Fibrosis Stage
Viral Load	r	1.000		
	p			
Beta-Klotho (nmol/L)	r	0.029	1.000	
	p	0.813		
Fibrosis Stage	r	0.153	0.011	1.000
	p	0.359	0.949	
PLR	r	-0.310	-0.100	-0.585
	p	0.011	0.420	< 0.001
NLR	r	-0.182	0.197	-0.357
	p	0.140	0.109	0.028
LMR	r	-0.063	-0.269	0.103
	p	0.610	0.028	0.539
PNR	r	-0.148	-0.322	-0.255
	p	0.232	0.008	0.122
MPV/PLT	r	0.127	0.006	0.391
	p	0.305	0.965	0.015
PDW/PLT	r	0.094	-0.015	0.399
	p	0.448	0.905	0.013
SII	r	-0.276	0.140	-0.643
	p	0.024	0.258	< 0.001
SIRI	r	-0.077	0.389	-0.201
	p	0.537	0.001	0.225
AAR	r	-0.134	-0.108	-0.036
	p	0.281	0.385	0.829
API	r	0.075	0.173	0.546
	p	0.548	0.161	< 0.001
APRI	r	0.491	0.153	0.425
	p	< 0.001	0.216	0.008
AAR/PLT	r	-0.013	-0.045	0.225
	p	0.918	0.717	0.174
GAPI	r	0.273	0.270	0.564
	p	0.025	0.027	< 0.001
AGR	r	0.162	-0.078	-0.286
	p	0.190	0.532	0.082
APGA	r	0.048	0.084	0.014
	p	0.703	0.503	0.933
PAPAS	r	-0.415	-0.178	-0.429
	p	0.001	0.153	0.008

Abbreviations: AAR – AST-to-ALT ratio, AGR – AST-to-GGT ratio, APGA – AST / platelet count / GGT / AFP, API – age-platelet index, APRI – AST-to-PLT ratio index, GAPI – GGT-to-PLT ratio index, LMR – lymphocyte-to-monocyte ratio, MPV – mean platelet volume, NLR – neutrophil-to-lymphocyte ratio, PAPAS – Platelet/ Age/ ALP/ AFP/ AST, PDW – platelet distribution width, PLR – platelet-to-lymphpcyte ratio, PLT – platelet count, PNR – platelet-to-neutrophil ratio, SII – systemic immune-inflammation index, SIRI – systemic inflammation response index.

Table 4. Sensitivity and specificity values of β-Klotho in the detection of hepatitis B disease

β-Klotho (nmol/L)	Sensitivity	Specificity
1.572	0.955	0.055
1.995	0.896	0.222
10.47	0.463	0.888
11.525	*0.463*	*0.944*
20.722	0.269	1.000

Characteristics of the test at the 11.525 nmol/L cut-off: AUC 0.674 (95% CI: 0.551-0.797), p=0.024.

In chronic HBV infection, the host immune response attacks infected hepatocytes, causing continuous cell injury and regeneration. Activated membrane proteases, such as ADAM17, can cleave transmembrane β-Klotho, releasing it into the circulation. Consequently, elevated serum β-Klotho may serve as a damage-associated biomarker reflecting hepatocellular injury, membrane disruption, and apoptosis. This hypothesis is supported by the statistically significant positive correlation observed between β-Klotho and the systemic inflammatory response index (SIRI; r=0.389, *p*=0.001), an established marker of systemic inflammation and liver damage [17].

We also observed a significant positive correlation between β-Klotho and GAPI, a modified ratio of GGT to platelet count. GAPI has been proposed as a useful non-invasive marker of hepatic inflammation and fibrosis, with some studies suggesting it may outperform APRI and FIB-4 in chronic HBV infection [18]. In contrast, no significant correlation was observed between β-Klotho and APRI, indicating that β-Klotho may primarily reflect active hepatocellular damage rather than cumulative fibrotic burden.

Our data suggest that serum β-Klotho levels are more reflective of ongoing liver injury and inflammation than

established fibrosis. Patients with early-stage fibrosis experiencing active hepatocyte damage may show elevated serum β-Klotho, while those with quiescent, advanced-stage fibrosis may have lower levels. ROC curve analysis revealed that β-Klotho has limited but statistically significant diagnostic utility for chronic HBV infection (AUC=0.674, *p*=0.024), with high specificity (94.4%) but low sensitivity (46.3%) at the optimal cut-off. This indicates that elevated β-Klotho strongly suggests disease presence, but normal levels cannot reliably exclude it. In comparison, studies in hepatocellular carcinoma report higher AUC values (0.862), suggesting that β-Klotho's diagnostic utility increases in advanced disease stages [15]. The absence of a significant correlation between β-Klotho levels and fibrosis stage is a notable finding. Liver fibrosis in chronic HBV infection is a progressive process driven by sustained inflammation, hepatocyte injury, and extracellular matrix remodeling. Biomarkers that reliably reflect fibrosis are expected to correlate with histological stage. However, our data indicate that β-Klotho does not meet this criterion. This observation is consistent with experimental and clinical studies reporting reduced hepatic Klotho expression in chronic liver injury, while circulating levels may not accurately mirror tissue expression [19,20]. It has been suggested that increased serum Klotho concentrations may result from enhanced shedding of membrane-bound Klotho in response to cellular stress or injury rather than reflecting fibrotic burden itself [15,20].

Importantly, while β-Klotho was not associated with fibrosis severity, several established hematological and composite indices demonstrated significant differences between groups and showed meaningful correlations with fibrosis stage. Platelet-related indices, including the platelet-to-lymphocyte ratio (PLR), MPV/ platelet ratio, and PDW/ platelet ratio, differed significantly across study groups and correlated with fibrosis stage. In particular, the moderate negative correlation between fibrosis stage and PLR observed in our cohort aligns with previous reports highlighting the role of platelet consumption and dysfunction in progressive liver fibrosis and portal hypertension. Platelets are increasingly recognized as active participants in hepatic inflammation and fibrogenesis, rather than passive bystanders [21,22]. Similarly, inflammation-based indices such as the systemic immune-inflammation index (SII) and neutrophil-to-lymphocyte ratio (NLR) exhibited significant correlations with fibrosis stage. These findings support the concept that immune dysregulation and systemic inflammation contribute to fibrosis progression in chronic HBV infection [23,24]. Composite fibrosis scores, including API, APRI, GAPI, and PAPAS, which integrate platelet counts and liver enzyme levels, demonstrated moderate correlations with histological fibrosis stage in our study. These results are consistent with previous studies showing that such indices effectively reflect cumulative necroinflammatory activity, hepatocellular injury, and portal hypertension [25]. Notably, the lack of correlation between β-Klotho and these fibrosis-related indices further supports the notion that β-Klotho is not a surrogate marker of fibrosis severity. Instead, the observed trend toward higher β-Klotho levels in patients with higher viral loads, although not statistically significant, may indicate a role for β-Klotho as a damage-associated or stress-responsive biomarker. Chronic HBV infection is characterized by ongoing immune-mediated hepatocyte injury, oxidative stress, and metabolic alterations, all of which may influence Klotho shedding and release into the circulation [26]. Taken together, our findings suggest that serum β-Klotho should not be interpreted as a marker of fibrosis progression in chronic HBV infection. In contrast, hematological indices and composite fibrosis scores demonstrated robust associations with histologically confirmed fibrosis stage, reinforcing their clinical utility. β-Klotho may instead reflect hepatocellular injury or systemic stress responses rather than structural fibrotic changes. Further longitudinal studies integrating serum measurements with tissue-level Klotho expression are warranted to clarify the biological and clinical significance of β-Klotho in chronic HBV infection.

Study limitations

Prospective, large-scale, multicenter studies are needed to monitor patients' clinical progress over time. This would enable us to determine whether changes in serum β-klotho levels have a prognostic value with regard to the natural history of the disease, as well as the development of cirrhosis and HCC. Investigating the effect of antiviral therapy on serum β-klotho levels could also help to answer the question of whether β-klotho could be used as a biomarker to monitor treatment response. Furthermore, the cross-sectional design of this study limits causal inferences between serum β-Klotho levels and disease progression. A limitation of the present study is that β-Klotho concentrations were reported in nmol/L according to the manufacturer's assay calibration. Since most previous studies report β-Klotho levels in pg/mL or ng/mL, direct comparison of absolute values is limited, although this does not affect within-group or between-group comparisons.

CONCLUSIONS

In the literature, serum β-klotho levels in patients with chronic hepatitis B virus (HBV) infection have been determined quantitatively using the enzyme-linked immunosorbent assay (ELISA), which is cheaper, more accessible, and simpler than molecular methods, although it does not replace virological assessment [27]. Our study

findings suggest that serum β-Klotho levels tend to increase with viral load in chronic HBV infection and may indicate liver damage. However, serum β-Klotho levels alone demonstrated limited diagnostic value in chronic HBV infection and no direct correlation was found between fibrosis stage and serum β-Klotho levels. In summary, β-Klotho may serve as a novel non-invasive indicator of hepatocellular injury in chronic HBV infection. Our results support the hypothesis that high serum β-Klotho levels reflect hepatocellular damage and membrane shedding rather than hepatic synthesis. Although serum β-Klotho levels increased across viral load categories, the lack of a significant correlation suggests that β-Klotho reflects ongoing cellular injury rather than viral replication itself. Therefore, β-Klotho may complement existing fibrosis indices by reflecting active hepatocellular injury and inflammatory activity, particularly in early or non-advanced stages of chronic HBV infection.

ABBREVIATIONS

AAR – AST-to-ALT ratio
AFP – alpha-fetoprotein
AGR – AST-to-GGT ratio
ALP – alkaline phosphatase
ALT – alanine aminotransferase
AST – aspartate aminotransferase
APGA – AST / platelet count / GGT / AFP
API – age-platelet index
APRI – AST-to-PLT ratio index
CRP – C-reactive protein
GAPI – GGT-to-PLT ratio index
GGT – gamma-glutamyl transferase
HBV – hepatitis B virus
LDH – lactate dehydrogenase
LMR – lymphocyte-to-monocyte ratio
MPV – mean platelet volume
NLR – neutrophil-to-lymphocyte ratio
PAPAS – Platelet/ Age/ ALP/ AFP/ AST
PCR – polymerase chain reaction
PDW – platelet distribution width
PLR – platelet-to-lymphpcyte ratio
PLT – platelet count
PNR – platelet-to-neutrophil ratio
SII – systemic immune-inflammation index
SIRI – systemic inflammation response index
WBC – white blood cell count

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AUTHORS' CONTRIBUTION

BE: conceptualization, methodology, analysis, data curation, writing- original draft, writing- review & editing.

HSE: conceptualization, methodology, analysis, data curation.

EK: conceptualization, methodology, analysis, data curation.

MO: conceptualization, methodology, analysis, data curation.

All authors have read and approved the final version of the manuscript.

CONFLICT OF INTEREST

None to declare.

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