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Original Article

Characterization of inflammatory protein expression patterns and their association with viral DNA load in hepatitis B virus infection via Olink proteomics analysis

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Abstract: Objectives: To investigate the differential expression of inflammatory proteins in the sera of patients with chronic hepatitis B (CHB) using Olink Targeted Proteomics and to explore their correlations with viral deoxyribonucleic acid (DNA) load. Methods: This retrospective study included 66 CHB patients and 22 healthy controls, with medical records collected between January and December 2023 at Nanning Customs, China. Viral DNA loads were quantified using real-time polymerase chain reaction (PCR), and 92 inflammatory proteins were profiled using Olink Targeted Proteomics. Results: A total of 38 proteins were differentially expressed between CHB patients and healthy controls, of which 7 were upregulated and 31 were downregulated. Correlation analysis revealed that viral DNA load was positively associated with the expression of osteoprotegerin (OPG) (P = 0.021) and chemokine (C-X-C motif) ligand 9 (CXCL9) (P = 0.002), and negatively associated with interleukin-10 (IL-10) (P = 0.007), cluster of differentiation 40 (CD40) (P = 0.004), and caspase-8 (CASP8) (P = 0.020). Functional enrichment analysis indicated that these proteins were mainly enriched in neutrophil chemotaxis, granulocyte migration, chemokine signaling pathways, cytokine activity, chemokine activity receptors, and tumor necrosis factor (TNF) receptors. Conclusions: Specific inflammatory proteins, including OPG, CXCL9, IL-10, CD40, CASP8, are associated with viral DNA load in CHB patients. These findings enhance the proteomic understanding of HBV pathogenesis and may offer potential therapeutic targets and biomarkers.

Keywords: Hepatitis B virus, proteomics, inflammatory factors, bioinformatics analysis

Introduction

The progression of chronic hepatitis B (CHB) involves a complex interplay of oxidative stress, disrupted lipid metabolism, hepatic lipid accumulation, and insulin resistance, which collectively trigger hepatic inflammatory responses, ultimately leading to systemic inflammation. Notably, these inflammatory processes frequently coexist with oxidative stress, exacerbating hepatocellular injury [1-3]. Inflammatory cytokines secreted by immune and certain non-immune cells (such as fibroblasts and vascular endothelial cells), including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), IL-8, cytokeratin 18 (CK18), chemerin, and chemokine (C-X-C motif) ligand 16 (CXCL16), play pivotal roles in orchestrating the immune response during CHB [4-7]. Previous studies have demonstrated that the viral load in HBV patients is positively correlated with the levels of interferon-gamma (IFN-γ) and IL-4, indicating that HBV replication intensity directly influences inflammatory activity [8].

Previous investigations have primarily focused on individual or a limited subset of cytokines, lacking a comprehensive proteomic assessment. Traditional proteomic analyses - based on protein separation and mass spectrometry - are often limited by sensitivity and throughput. The Olink multiplex proximity extension assay (PEA) has recently emerged as a powerful tool in precision proteomics research, providing a novel option for multi-omics studies. This technology efficiently and accurately detects a mul-

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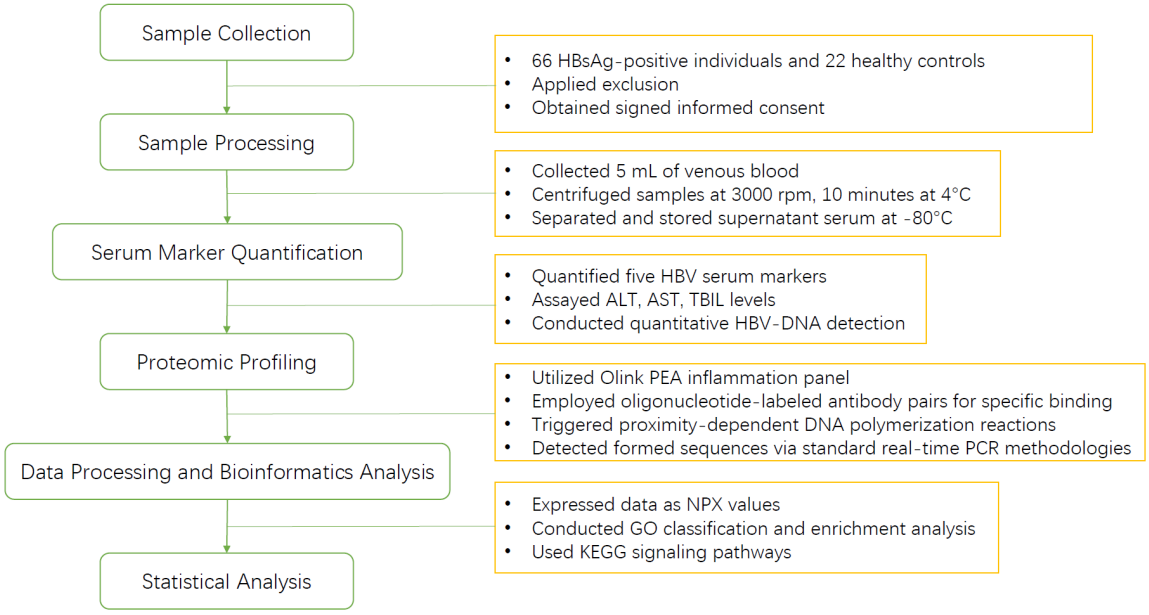

Figure 1. Flow chart. HBV: hepatitis B virus; HBsAg: hepatitis B surface antigen; ALT: alanine aminotransferase; AST: aspartate aminotransferase; TBIL: total bilirubin; PEA: proximity extension assay; NPX: Normalization Protein Expression; GO: gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes.

titude of low-abundance biomarkers that are difficult to quantify using traditional mass spectrometry or microarray technologies [9-11].

In this study, we utilized the Olink targeted proteomics platform to systematically profile serum inflammation associated proteins in individuals with HBV infection. This study aimed to (1) characterize the differential expression patterns of inflammation associated protein in CHB patients, (2) investigate the correlation between these proteomic expressions and HBV DNA loads, and (3) identify key proteins involved in the immunopathogenesis of HBV, thereby contributing to a deeper understanding of the disease process and potential therapeutic targets.

Materials and methods

Patients and samples

This retrospective study included 66 individuals with positive serum hepatitis B surface antigen (HBsAg), based on medical records of entry-examinees at Nanning Customs in Guangxi, China, between January and December 2023. An additional 22 healthy individuals from the same period were recruited as controls. Exclusion criteria included co-infection with other hepatitis viruses, moderate-to-severe hepatic steatosis, alcoholic liver disease, liver cancer or other malignancies, cirrhosis, or severe cardio-pulmonary, renal, or endocrine diseases. Ethical approval for this study was granted by the Medical Ethics Committee of the First Affiliated Hospital of Guangxi Medical University (Approval Number: 2023-SW030-01). A schematic flowchart of the study design is shown in **Figure 1**.

Five milliliters (mL) of fasting venous blood were drawn from each participant via the antecubital vein. Samples were centrifuged at 3,000 revolutions per minute (rpm) for 10 minutes at 4°C, and the resulting serum supernatant was collected. Aliquots of 100 µL were stored at -80°C for subsequent proteomic analysis, while the remaining serum was used for HBV DNA viral load detection. A DNA viral load > 102 IU/mL was considered indicative of HBV-DNA positivity.

Quantitative detection of serum markers

Quantitative detection of five HBV serological markers, including HBsAg, hepatitis B surface antibody (HBsAb), hepatitis B e antigen (HBeAg), hepatitis B e antibody (HBeAb), and hepatitis B core antibody (HBcAb), was per-

formed using a chemiluminescent immunoassay (CLIA) on the AutoLumo A2000 plus analyzer (Antu, Zhengzhou, China). Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total bilirubin (TBIL) were measured using the AU5800 fully automated biochemical analyzer (Beckman). Quantitative detection of HBV-DNA was conducted using the CFX96™ real-time fluorescence-based quantitative polymerase chain reaction (PCR) system, with a commercial HBV-DNA detection kit (Da'an, Zhongshan, China). All procedures followed the manufacturer's protocol. An HBV DNA concentration > 10^2 copies/ml was considered positive.

Proteomic profiling of soluble factors in plasma

Plasma samples were analyzed using the Olink multiplex proximity extension assay (PEA) inflammation panel, which targets 92 immune-related proteins (IRPs) (Olink Bioscience AB, Shanghai, Jingzhou, China). This technology employs oligonucleotide-labeled antibody pairs that bind specifically to target proteins. Proximal binding of these antibody pairs triggers a proximity-dependent DNA polymerization reaction, generating a novel PCR target sequence, which is subsequently quantified by real-time PCR.

The Olink platform has demonstrated high analytical performance, with validation studies, both by the manufacturer and independent researchers, reporting excellent accuracy (correlation coefficients > 0.9 with ELISA), intra- and inter-assay coefficients of variation (CVs) below 10%, and high reproducibility across laboratories [12-14]. Protein concentrations were expressed as normalized protein expression (NPX) values, exhibiting similar distributions to those of log2-transformed.

Olink PEA method was chosen for its capacity to simultaneously quantify multiple low-abundance proteins with high sensitivity and specificity. Unlike traditional ELISA, which is limited in multiplexing capability and throughput, the Olink platform enables comprehensive profiling of inflammatory mediators essential for large-scale proteomic analyses.

Bioinformatics analysis

Experimental results were presented as Normalization Protein Expression (NPX) values, which represent relative quantification derived from log-transformed cycle threshold (CT) values obtained via qPCR. These NPX values were utilized for further downstream bioinformatics analysis. Gene Ontology (GO) classification and enrichment analysis were conducted using the GO database (http://www.geneontology.org), and results were visualized as bar charts and bubble plots. Additionally, signaling pathway enrichment analysis was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.kegg.jp), with graphical outputs similarly presented as bar charts and bubble plots.

Primary and secondary outcomes

The primary outcome was the identification and characterization of differentially expressed inflammatory proteins between CHB patients and healthy controls using the Olink proteomics platform.

The secondary outcomes included GO functional enrichment and KEGG pathway enrichment analyses of the identified differentially expressed proteins, as well as quality control (QC) assessments of serum specimens to ensure data reliability and reproducibility.

Statistical analysis

Proteomic profiling of soluble factors was analyzed using Olink NPX Signature software (version 1.5.3.0). Differentially expressed proteins between CHB patients and healthy controls were identified based on NPX values, with statistical significance set at $P < 0.05$. Proteins with positive NPX differences were classified as upregulated, while those with negative differences were considered downregulated.

Further statistical analyses were performed using SPSS 26.0. The Pearson correlation test was applied to assess correlations between two continuous variables, following confirmation of normal data distribution. A two-sided P value < 0.05 was considered statistically significant.

Results

Characteristics of study participants

A total of 88 subjects were included in this study, comprising 66 patients with CHB and 22

Table 1. Demographic characteristics of study subjects

Variable	CHB	Healthy control	t/χ^2	*P*-value
n	66	22		
Age (year, $\bar{X}\pm S$)	41.58±8.37	39.23±6.70	1.193	0.236
Gender male, n (%)	59 (89.4)	17 (77.3)	2.058	0.151
BMI (kg/m^2, $\bar{X}\pm S$)	22.88±2.76	22.69±2.38	0.290	0.773
Smoking history, n (%)	16 (24.24%)	5 (22.73%)	0.021	0.885
Drinking history, n (%)	20 (30.3%)	4 (18.18%)	1.222	0.269
Marital Status (Married/Unmarried or Divorced), n (%)	54 (81.82%)/12 (18.18%)	19 (86.36%)/3 (13.64%)	0.027	0.870

Notes: CHB: chronic hepatitis B; BMI: body mass index.

Table 2. Baseline characteristics of CHB patients

Variable	CHB
n	66
HBV-DNA (IU/ml, $\bar{X}\pm S$)	(9.16±3.51)×10^6
Duration of HBV infection (year, $\bar{X}\pm S$)	13.27±4.16
ALT (IU/L)	51.22 (32.62-98.26)
AST (IU/L)	37.67 (18.22-66.52)
TBIL (μmol/L)	12.63 (10.82-17.71)

Notes: CHB: chronic hepatitis B; HBV: hepatitis B virus; DNA: deoxyribonucleic acid; ALT: alanine aminotransferase; AST: aspartate aminotransferase; TBIL: total bilirubin.

Table 3. HBV serum marker levels and HBV-DNA load in CHB patients

HBV markers	N	HBV-DNA load (n)		
		< 10^2 IU/ml	10^2-10^4 IU/ml	> 10^4 IU/ml
HBsAg				
positive	66	22	22	22
HBeAg				
positive	7	2	2	3
negative	59	20	20	19
ALT				
positive	26	2	2	22
negative	40	20	20	0

Notes: HBV: hepatitis B virus; DNA: deoxyribonucleic acid; HBsAg: hepatitis B surface antigen; HBeAg: hepatitis B e antigen; ALT: alanine aminotransferase; CHB: chronic hepatitis B.

healthy controls. No statistically significant differences were observed between the two groups in terms of age, sex, body mass index (BMI), smoking history, alcohol consumption, or marital status (P > 0.05).

The mean HBV DNA load in the CHB group was 9.16±3.51×10^6 IU/ml, and the average duration of HBV infection was 13.27±4.16 years. Median ALT and AST levels were 51.22 IU/L (interquartile range [IQR]: 32.62-98.26 IU/L) and 37.67 IU/L (IQR: 18.22-66.52 IU/L), respectively. The median TBIL level was 12.63 μmol/L (IQR: 10.82-17.71 μmol/L) (**Tables 1**, **2**).

HBV serum markers (HBsAg, HBeAg, ALT) and DNA load detection in CHB patients

All 66 CHB patients tested positive for HBsAg. Among them, 44 patients demonstrated HBV-DNA positivity. Within this subset, 5 patients were concurrently positive for both HBeAg and HBV-DNA, whereas 39 were negative for HBeAg but positive for HBV-DNA. Furthermore, 26 patients were ALT-positive, with 84.62% (22 patients) had HBV DNA loads surpassing 10^4 IU/ml (**Table 3**).

Quality control of serum biological specimens

QC assessments were conducted on all 88 serum samples subjected to Olink proteomics analysis, and the QC results are shown in **Figure 2**. Of these, 84 samples met the predefined QC criteria, whereas four samples (E136, E145, E222, and E242) were flagged as QC alerts and excluded from subsequent analyses. Following the exclusion of these four samples, no statistically significant disparities were noted in gender or age between the CHB and control groups (P > 0.05).

Detection of serum inflammatory protein expression levels between the HBV-infected and healthy control groups

The expression levels of 92 inflammatory proteins were measured in serum samples from

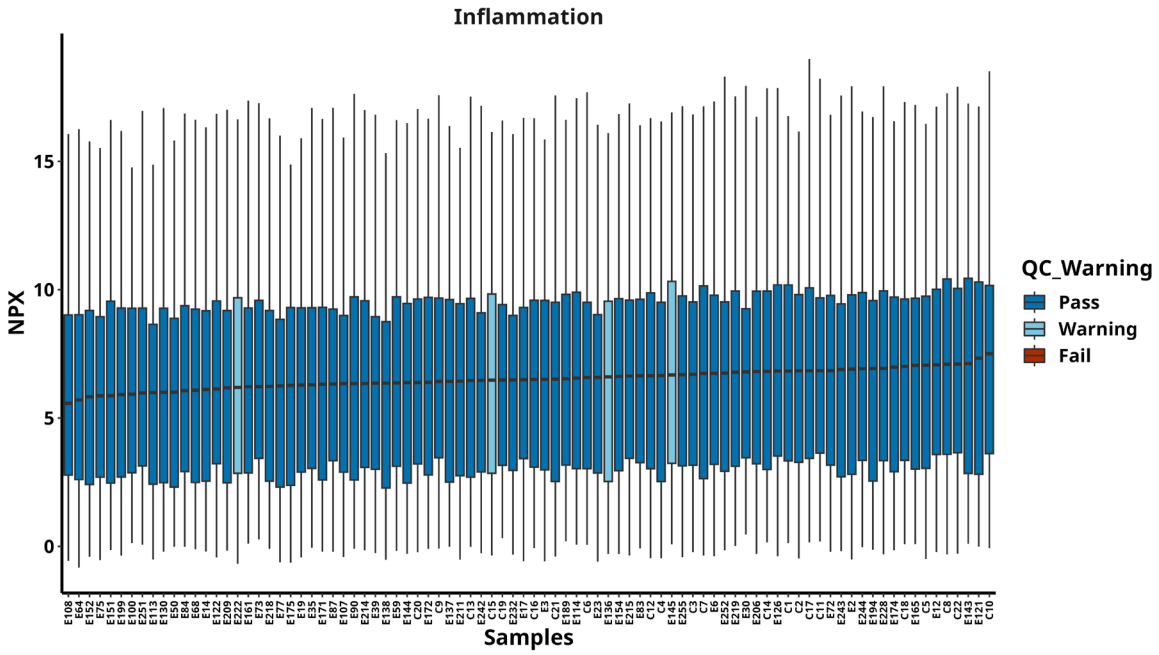

Figure 2. Inclusion criteria based on quality control outcomes of serum samples. The horizontal axis represented the sample names, while the vertical axis denoted the NPX values. The light blue color indicated samples flagged for QC alerts and the dark blue color represented samples that had successfully passed the QC criteria. QC: quality control; NPX: Normalization Protein Expression.

both groups. Cluster analysis was performed on the obtained NPX values, and the results are presented in **Figure 3**.

A total of 38 differentially expressed proteins were identified based on mean NPX value comparisons between the two groups. Among these, 7 proteins were significantly upregulated, sorted by ascending *P* values: cub domain-containing protein 1 (CDCP1), latency-associated peptide transforming growth factor-beta-1 (LAP TGF-beta-1), IL-10, interleukin-15 receptor alpha (IL-15RA), CXCL9, OPG, and tumor necrosis factor receptor superfamily member 9 (TNFRSF9). In contrast, 31 proteins were significantly downregulated, sorted by ascending *P* values: axis inhibition protein 1 (AXIN1), oncostatin m (OSM), IL-8, transforming growth factor-alpha (TGF-alpha), stanniocalcin-1 (ST1A1), signal transducing adapter molecule-binding protein (STAMBP), sirtuin 2 (SIRT2), tumor necrosis factor superfamily member 14 (TNFSF14), CD40, c-c motif chemokine ligand 28 (CCL28), c-x-c motif chemokine ligand 5 (CXCL5), c-x-c motif chemokine ligand 6 (CXCL6), high mobility group box 1 (HMGB1, also known as EN-RAGE), c-c motif chemokine ligand 3 (CCL3), fibroblast growth factor 23 (FGF-23), c-c motif chemokine ligand 4 (CCL4), monocyte chemoattractant protein-3 (MCP-3), adenosine deaminase (ADA), tumor necrosis factor-related activation-induced cytokine (TRANCE), c-x-c motif chemokine ligand 1 (CXCL1), CASP8, interleukin-12 subunit beta (IL-12B), monocyte chemoattractant protein-4 (MCP-4), beta-nerve growth factor (Beta-NGF), interleukin-5 (IL-5), tnf-related apoptosis-inducing ligand (TRAIL), IL-6, stem cell factor (SCF), hepatocyte growth factor (HGF), and interleukin-2 receptor subunit beta (IL-2RB).

GO functional enrichment of differential proteins between the HBV and healthy control groups

GO functional enrichment analysis was conducted on the seven significantly upregulated proteins and 31 downregulated proteins identified between the HBV and control groups. The results indicated that these differentially expressed proteins were primarily enriched in biological processes such as neutrophil chemotaxis, granulocyte migration, and chemotaxis, and chemokine-mediated signaling pathways. In terms of molecular function categories, enrichment was observed in cytokine activity,

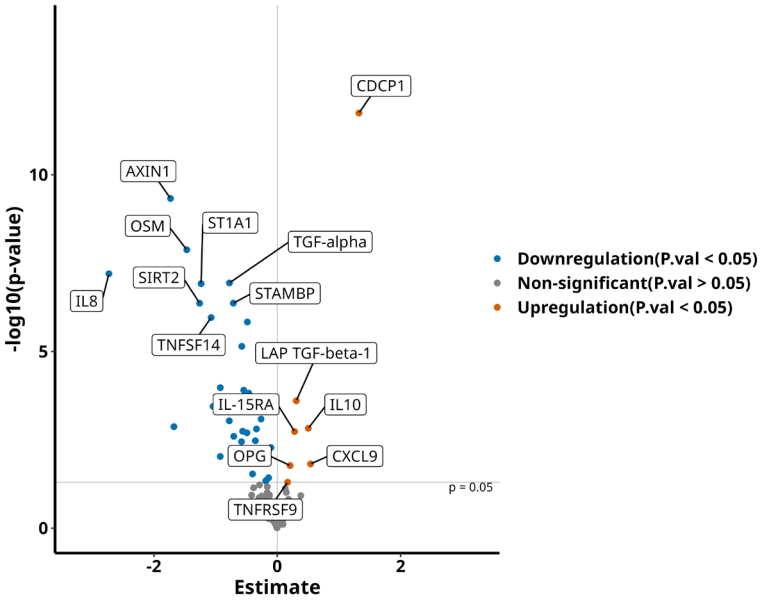

Figure 3. Differentially expressed inflammation-related proteins between the HBV and healthy control groups. The blue points represented downregulated differentially expressed proteins, while the orange points indicated upregulated differentially expressed proteins. CDCP1: cub domain-containing protein 1; LAP TGF-beta-1: latency-associated peptide transforming growth factor-beta-1; IL-10: interleukin-10; CXCL9: chemokine (C-X-C motif) ligand 9; OPG: osteoprotegerin; TNFRSF9: tumor necrosis factor receptor superfamily member 9; AXIN1: axis inhibition protein 1; OSM: oncostatin m; TGF-alpha: transforming growth factor-alpha; ST1A1: stanniocalcin-1; SIRT2: sirtuin 2; TNFSF14: tumor necrosis factor superfamily member 14; IL-8: interleukin-8; STAMBP: signal transducing adapter molecule-binding protein; IL-15RA: interleukin-15 receptor alpha.

chemokine activity and receptor binding, and tumor necrosis factor receptor activity (**Figure 4**).

KEGG pathway of differential proteins between the HBV-infected group and the healthy control group

KEGG pathway analysis was conducted on the same set of differentially expressed proteins. The results revealed significant enrichment in pathways such as cytokine-cytokine receptor interaction, viral protein interaction with cytokine and cytokine receptor, Toll-like receptor signaling, and chemokine signaling pathways (**Figure 5**).

Correlation between HBV-DNA load and differential protein expression in CHB patients

Pearson bivariate correlation analysis was conducted to assess the relationship between HBV-DNA load levels and the expression levels of the 38 differentially expressed proteins in the CHB group (**Table 4**). The results revealed significant positive correlation between HBV-DNA load and the upregulated proteins OPG and CXCL9, as well as significant negative correlations with the downregulated proteins IL-10, CD40, and CASP8 ($P < 0.05$).

To further quantify the strength of these relationships, effect sizes were calculated based on the squared Pearson correlation coefficients. Multivariate regression analysis, adjusted for potential confounders, confirmed the observed associations. Specifically, HBV-DNA load remained positively correlated with OPG ($\beta = 0.267$) and CXCL9 ($\beta = 0.359$) and negatively correlated with IL-10 ($\beta = -0.312$), CD40 ($\beta = -0.289$), and CASP8 ($\beta = -0.254$).

Correlation between inflammatory protein levels and other HBV infection markers

CXCL9 expression showed a significant positive correlation with the duration of HBV infection ($P < 0.05$), while IL-10 and OPG did not reach statistical significance ($P > 0.05$) (**Table 5**). Regarding HBeAg status, CXCL9 and OPG levels were significantly elevated in HBeAg-positive patients, indicating positive correlations. Conversely, IL-10, CD40, and CASP8 levels were significantly reduced in relation to HBeAg levels, suggesting negative correlations with HBeAg positivity ($P < 0.05$).

Discussion

Upon hepatitis B virus (HBV) infection, individuals typically undergo four distinct immunological stages: immune tolerance, immune clearance, immune control, and immune reactivation. During the immune control phase, patients generally present with minimal clinical manifestations, accompanied by mild inflammation in the liver parenchyma and limited inflammatory necrosis or fibrosis within the

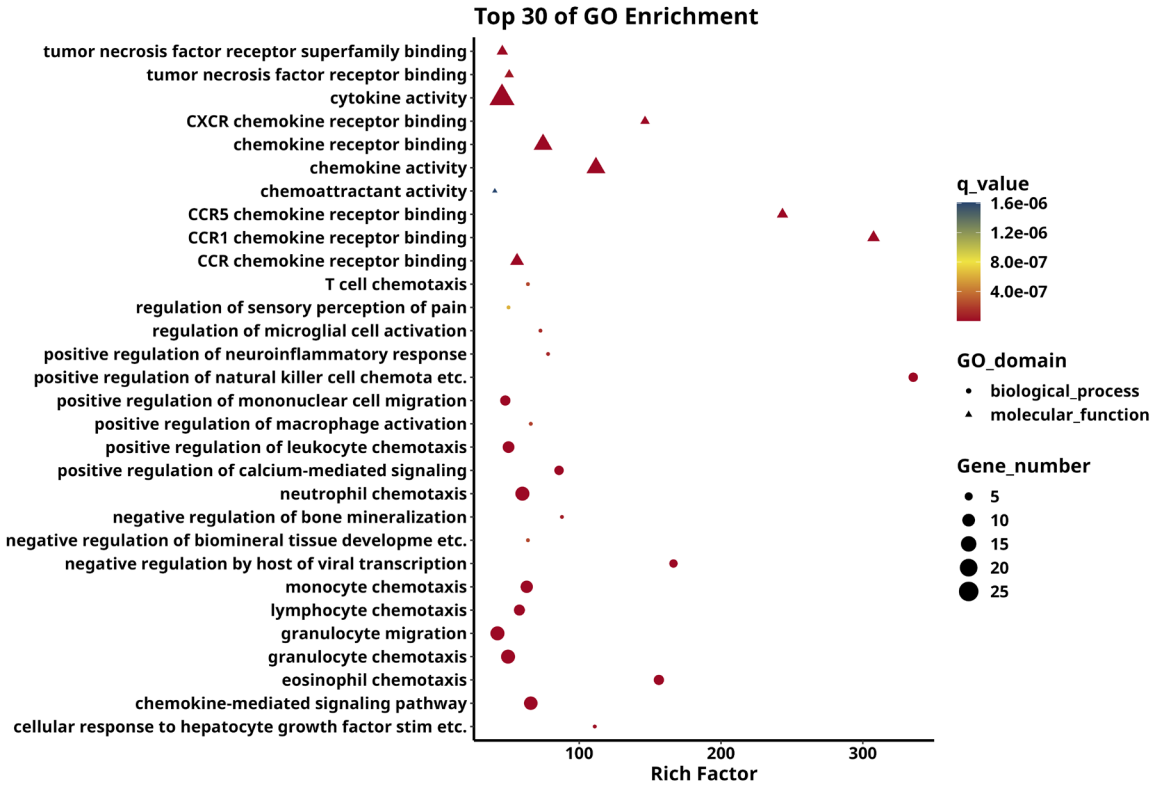

Figure 4. Bubble plot of GO pathway enrichment analysis for differential protein-coding genes between the HBV and healthy control groups. The x-axis signified the Rich factor, representing the count of differential proteins within each GO term. The y-axis depicted the GO Term, indicating functional annotation. Point size scaled with protein quantity, while color intensity reflected enrichment significance ($P < 0.05$ indicating statistical significance). This figure highlighted the top 30 most significantly enriched functions. GO: gene ontology; CXCR: C-X-C motif chemokine receptor; CCR: C-C motif chemokine receptor.

portal tracts and adjacent tissues [15]. However, in the immune reactivation phase, liver biopsy often reveals pronounced inflammation, moderate to severe inflammatory necrosis, and fibrosis in portal tracts and surrounding tissues [5]. Initially, the host immune response effectively suppress HBV infection and replication by overcoming early immune tolerance. However, persistent antigenic stimulation may exhaust HBV-specific cellular immunity, resulting in a shift toward nonspecific inflammatory injury as the primary immune-mediated response to viral replication [16].

Consistent with the established immunological staging of chronic HBV infection [15], our study classified 20 patients (30.30%) in the immune control phase, 24 (36.36%) in the immune reactivation phase, 19 (28.79%) in the indeterminate phase, and 3 (4.55%) in the immune clearance phase. This distribution highlights the complexity and heterogeneity of chronic HBV progression. Due to limitations in study design and clinical constraints, invasive liver biopsy was not performed. Instead, non-invasive biomarkers were used to assess disease stages. Although inflammatory necrosis is a hallmark of the immune clearance and reactivation phases, the relationship between this injury and serum inflammatory protein factors remains incompletely understood. Our study aimed to elucidate the expression patterns of these proteins, providing insights into their potential mechanistic roles in disease progression.

Overexpression of both pro-inflammatory and anti-inflammatory cytokines contributes to hepatic inflammation. Key pro-inflammatory cytokines include IL-1β, IL-6, IL-8, and TNF-α. Yasir et al. utilized Olink inflammatory proteomics and identified 27 proteins associated with fatty liver, among which 15 were linked to liver fibrosis, including upregulated expression of IL-18R1

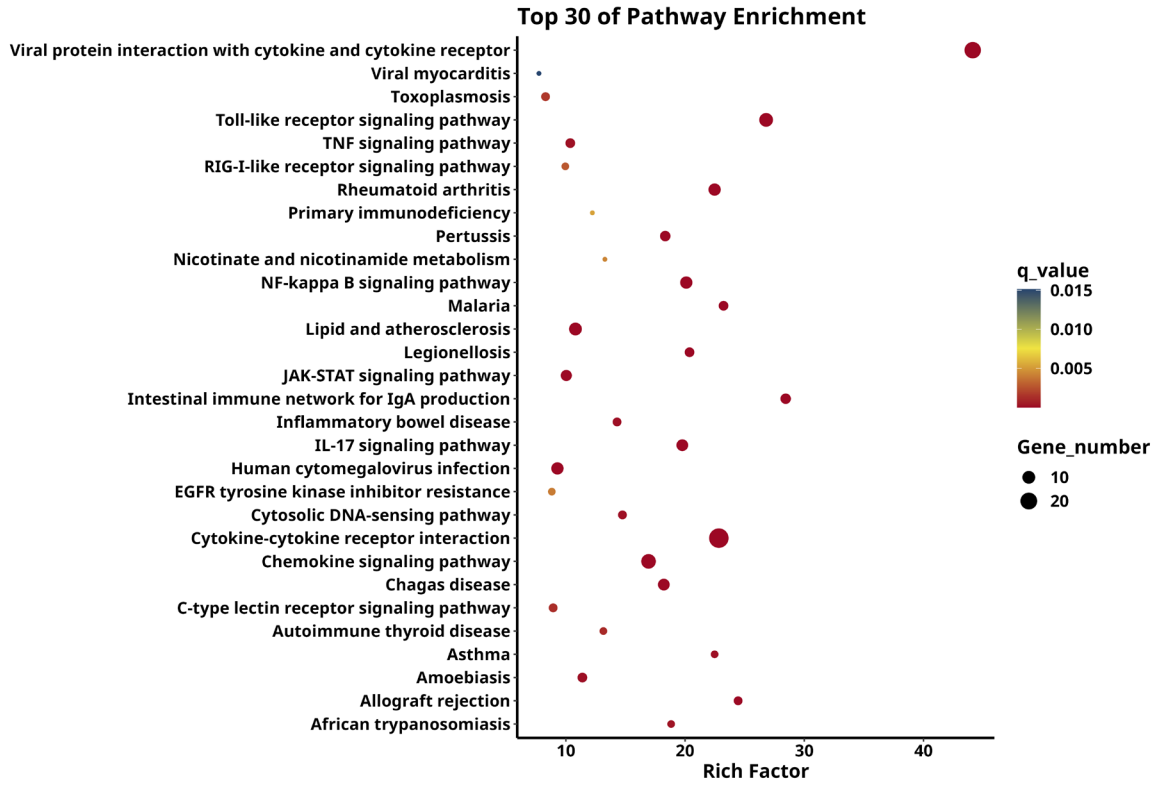

Figure 5. KEGG pathway analysis of differential proteins between the HBV-infected group and the healthy control group. The x-axis represented the Rich factor, indicating the count of differentially expressed proteins within each KEGG pathway. The y-axis, labeled "Pathway", annotated the respective pathways. Point size was scaled with the number of proteins, while color intensity reflected the significance of enrichment, with $P < 0.05$ indicating statistical significance. This plot highlighted the top 30 most significantly enriched functions. TNF: tumor necrosis factor; NF-kappa B: nuclear factor kappa-light-chain-enhancer of activated b cells; JAK-STAT: janus kinase-signal transducer and activator of transcription; IL-17: interleukin-17; DNA: deoxyribonucleic acid; EGFR: epidermal growth factor receptor.

[17]. In patients with hepatocellular carcinoma, levels of granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-6, IL-8, and monocyte chemoattractant protein-1 (MCP-1) were positively correlated with postoperative liver injury [18]. Conversely, anti-inflammatory cytokines such as IL-4, IL-10, and transforming growth factor-β (TGF-β) exhibit hepatoprotective effects. Reduced serum IL-4 levels have been reported in CHB patients [19] and negatively correlates with serum HBV RNA, along with IL-10 [20]. Moreover, glutamate dehydrogenase (GLDH), IL-6, and IL-10 have been proposed as biomarkers of liver injury severity [21].

High-throughput proteomics technology identified 38 differentially expressed proteins between CHB patients and healthy controls, including 7 upregulated and 31 downregulated proteins. Notably, the anti-inflammatory cytokines IL-10 and LAP TGF-β-1 were unexpectedly upregulated, despite previous research reporting their downregulation in liver cancer. Conversely, the pro-inflammatory cytokines IL-6 and IL-8 were downregulated, contrasting with their persistent elevation in liver cancer as reported in prior studies [22]. However, a broader literature review indicates significant increases in IL-6, IL-10, and IL-17 in liver failure accompanied by sepsis [23], highlighting the dynamic interactions between pro-inflammatory and anti-inflammatory cytokines as disease progresses. These findings highlight the need for further investigation into the underlying mechanisms. Additionally, this study had a mean HBV-DNA load of $(9.16\pm3.51)\times10^6$ IU/ml, indicative of relatively low viral replication and the exclusion of acute or severe cases, which may partially explain the observed protein expression patterns.

Table 4. Correlation between HBV-DNA load and differential protein expression in CHB patients

Protein	Normalization Protein Expression (NPX, $\bar{X}$±S)	r	*P*-value	Effect Size (r^2)	β (Standardized Coefficient)
CXCL9	8.336±0.841	0.371	0.002	0.138	0.359
CD40	12.449±0.414	0.347	0.004	0.121	-0.289
IL-10	5.230±0.833	0.329	0.007	0.108	-0.312
CASP8	6.507±0.898	0.286	0.020	0.082	-0.254
OPG	11.467±0.371	0.284	0.021	0.081	0.267

Notes: CXCL9: chemokine (C-X-C motif) ligand 9; CD40: cluster of Differentiation 40; IL-10: interleukin-10; CASP8: caspase-8; OPG: osteoprotegerin; CHB: chronic hepatitis B.

Table 5. Correlation between inflammatory protein levels and other HBV infection markers

Protein	r	*P*-value
Correlation with Duration of HBV Infection		
CXCL9	0.425	0.038
IL-10	0.368	0.072
OPG	0.347	0.059
Correlation with HBeAg Levels		
CXCL9	0.315	0.005
CD40	0.287	0.014
IL-10	0.275	0.018
CASP8	0.253	0.029
OPG	0.246	0.032

Notes: CXCL9: chemokine (C-X-C motif) ligand 9; IL-10: interleukin-10; OPG: osteoprotegerin; CD40: cluster of Differentiation 40; CASP8: caspase-8.

GO and KEGG pathway analyses were performed on the 38 differentially expressed proteins. Molecular function enrichment revealed significant involvement in cytokine activity, chemokine activity, and receptor binding, as well as TNF receptor activity. KEGG pathway analysis demonstrated enrichment in cytokine-cytokine receptor interaction, viral protein-cytokine receptor interaction, Toll-like receptor signaling, and chemokine signaling pathways [24]. These findings are consistent with previous literature indicating that cytokines, including interleukins, interferons, TNF superfamily members, colony-stimulating factors, chemokines, and growth factors, play central roles in the pathogenesis of viral hepatitis, autoimmune liver diseases, drug-induced liver injury, hepatic fibrosis, and primary liver cancer [25-28]. While our study provides valuable insights into the functional roles of these proteins through GO and KEGG pathway analyses, further bioinformatics analyses such as protein-protein interaction (PPI) network construction and principal coordinate analysis (PCoA) were not conducted. Incorporating these analyses in future studies could enhance the understanding of molecular interactions among the proteins and clarify clustering patterns within the sample population.

Previous studies reported that CHB patients with significant histological liver damage exhibited lower HBV-DNA levels compared to those without such damage [29]. Additionally, reduced HBV-DNA levels correlated with more severe liver fibrosis [30]. In our study, 44 cases (two-thirds) had DNA loads < 10^4 IU/ml, suggesting substantial liver tissue damage. This aligns with our hypothesis, based on the natural course of chronic HBV infection, that the enrolled patients were in an active inflammatory state despite low viral replication.

The viral load of HBV in persistent infections has a direct impact on the severity of inflammation, exhibiting a positive correlation with the concentrations of IFN-γ and IL-4 [8]. In patients with CHB, a significant correlation has been observed between the expression of Wnt5a mRNA and HBV-DNA load, accompanied by elevated hepatic Wnt5a protein levels compared to healthy controls [31]. Furthermore, the HBV-DNA load is correlated with the level of TGF-β in both acute and chronic hepatitis B [32]. Our study aimed to investigate the correlation between HBV-DNA load and differential inflammatory protein expression using high-throughput proteomics. Results showed a positive correlation between HBV-DNA load and levels of OPG, CXCL9, IL-10, CD40, and CASP8. Notably, CXCL9 was elevated with prolonged

HBV infection duration, indicating its potential role in mediating sustained inflammation during chronic infection. Analysis of HBeAg status further revealed that CXCL9 and OPG were significantly upregulated in HBeAg-positive patients, whereas IL-10, CD40, and CASP8 were downregulated. These findings highlight the complex interplay between HBV markers and host inflammatory protein expression, which may collectively contribute to the immunopathogenesis of CHB. It is worth noting that IL-4 protein expression in CHB patients did not differ significantly from healthy controls, hence its correlation with HBV-DNA load was not assessed in this study. Furthermore, previous transcriptome studies revealed increased expression of macrophage, T-cell, immune-negative regulator, chemokine, and interferon-stimulated gene (ISG) marker genes during immune activation; however, these gene expression changes did not correlate positively with HBV-DNA levels [33, 34]. These findings indirectly support our hypothesis of a correlation between HBV-DNA load and inflammatory proteomics.

This study has several limitations. First, the sample size is relatively small, which limits the statistical power and generalizability of our findings. The small sample size may reduce the reliability of the correlation analysis between viral DNA load and inflammatory protein expression levels. Second, the single-center design may introduce center-specific bias, further restricting the extrapolation of results to broader populations. Third, the cross-sectional nature of our study makes it difficult to establish causal relationships between inflammatory protein expression and HBV-DNA load. Fourth, the proteomic analysis was limited to a predefined panel of 92 inflammatory proteins, which is insufficient to represent the full inflammatory proteomic profile in CHB. Additionally, ethnic differences could influence the interpretation of our results and their applicability to other ethnic groups. While we excluded co-infections with other hepatitis viruses and pre-existing liver diseases, other potential confounding factors such as diet, lifestyle, and genetic background were not fully considered. These factors could influence the expression of inflammatory proteins and the viral DNA load, potentially introducing bias.

Despite these limitations, our study provides valuable insights into the relationship between specific inflammatory proteins and CHB pathogenesis. Future studies should include larger, multi-center cohorts to enhance statistical robustness and population representativeness. A broader proteomic panel should be employed to capture the full spectrum of inflammatory mediators. Adopting a longitudinal study design will help elucidate dynamic changes and causal relationships over time. Furthermore, controlling confounding factors like diet, lifestyle, and genetic background will enhance the accuracy of observed relationships. These efforts will deepen our understanding of inflammatory mechanisms in CHB and their potential as therapeutic targets and biomarkers.

Conclusions

In conclusion, our study employed Olink-targeted proteomics to identify a panel of differentially expressed inflammatory proteins between CHB patients and healthy controls. This approach enabled the detection of low-abundance proteins that are often undetectable by conventional mass spectrometry or microarray techniques. Bioinformatics analysis elucidated the biological mechanisms of these proteins in CHB patients. Notably, OPG, CXCL9, IL-10, CD40, and CASP8 were closely related to HBV-DNA load. However, literature on the association of proteins like TRANCE and Beta-NGF with HBV infection is scarce. Future studies should aim to improve experimental design, expand sample size, and explore the mechanistic roles of these proteins in CHB progression, providing insights into the mechanisms of inflammatory proteins in CHB infection.

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Disclosure of conflict of interest

None.

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