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# CXCL16 gene promoter methylation is associated with inflammation in patients with chronic hepatitis B

**Zhezhe Tian**
Qilu Hospital of Shandong University
**Miaomiao Xu**
Qilu Hospital of Shandong University
**Hongfei Li**
Qilu Hospital of Shandong University
**Yunxin Yao**
Qilu Hospital of Shandong University
**Yuchen Fan**
Qilu Hospital of Shandong University
**Shuai Gao**
Qilu Hospital of Shandong University
**Kai Wang**
wangdoc876@126.com
Qilu Hospital of Shandong University

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# Abstract

Hepatitis B virus (HBV) infection causes persistent hepatic inflammation, and chemokine CXCL16 serves as a key mediator in liver injury, but its epigenetic regulation in chronic hepatitis B (CHB) remains unclear. This study enrolled 217 CHB patients (113 HBeAg-positive, 104 HBeAg-negative) and 27 healthy controls to investigate CXCL16 promoter methylation, mRNA and protein expression, as well as their correlations with inflammatory indices and liver injury markers. The results showed that CXCL16 promoter methylation was significantly lower, while CXCL16 mRNA and serum levels were markedly higher in CHB patients, especially in HBeAg-positive cases. CXCL16 promoter methylation was negatively correlated with CXCL16 expression, HBeAg status, ALT, AST, TNF-α and IL-1βlevels. ROC analysis indicated that CXCL16 promoter methylation had favorable diagnostic value for evaluating hepatic inflammatory injury in CHB patients. In conclusion, CXCL16 promoter hypomethylation is closely associated with elevated CXCL16 expression and exacerbated hepatic inflammation in CHB patients, and may serve as a potential non-invasive biomarker for assessing CHB-related liver inflammation. These findings enrich the epigenetic mechanism underlying HBV-related hepatic inflammation and provide a novel target which is CXCL16 for clinical evaluation and intervention.


# [Introduction]

Hepatitis B virus (HBV) infection represents a major global public health issue, as early-onset infection frequently progresses to lifelong chronic infection and persistent viral carriage (1, 2). Notably, the World Health Organization (WHO) estimates that the global seroprevalence of hepatitis B surface antigen (HBsAg) stands at 3.2% as of 2024, with 254 million individuals living with chronic HBV infection and approximately 820,000 deaths annually attributed to HBV-related liver diseases (3).

As the prototypical member of the Hepadnaviridae family, hepatitis B virus (HBV) is a hepatotropic, noncytopathic DNA virus transmitted via contact with infected blood and bodily fluids, which elicits immune-mediated liver diseases spanning diverse severity levels and clinical courses (4). Notably, HBV establishes persistent infection by generating a covalently closed circular DNA (cccDNA) intermediate within the nuclei of infected hepatocytes, alongside stably integrated viral sequences that serve as stable transcription templates for viral protein synthesis (5, 6). Crucially, this cccDNA minichromosome can maintain chronic HBV infection even in the setting of prolonged viral replication suppression mediated by polymerase inhibitors (7, 8). Persistent HBV infection triggers long-term imbalance in the hepatic immune microenvironment, characterized by massive infiltration of inflammatory cells, excessive release of pro-inflammatory mediators, and chronic low-grade inflammation, which gradually exacerbates hepatocellular damage, hepatic fibrosis, and even progresses to cirrhosis and hepatocellular carcinoma over time (9). Among the numerous immune regulatory molecules involved in this process, chemokines play a pivotal role in mediating immune cell recruitment, modulating inflammatory signal transduction, and shaping the pro-inflammatory microenvironment in the HBV-infected liver (10–13).

As a key member of this chemokine family, chemokine (C-X-C motif) ligand 16 (CXCL16) has emerged as a critical mediator of hepatic inflammation, supported by its high expression level in liver tissue and core role in immune cell recruitment and activation within the hepatic microenvironment (14, 15). CXCL16 presents in two distinct biologically active forms: transmembrane and soluble isoforms (16). The transmembrane isoform functions as a cell-surface adhesion molecule that accelerates the infiltration of immune cells into inflamed hepatic sites, while its proteolytic cleavage yields the soluble isoform, which acts as a specific chemoattractant targeting cells expressing CXC-chemokine receptor 6 (CXCR6); of note, CXCL16 is the sole endogenous ligand for CXCR6 (17, 18). The CXCL16-CXCR6 axis has also been demonstrated to exert vital functions in the pathogenesis of multiple inflammatory and fibrotic diseases beyond hepatic disorders (19–22). Growing evidence confirms elevated circulating and tissue CXCL16 levels in multiple acute and chronic liver diseases, including non-alcoholic fatty liver disease (NAFLD) and acute liver injury, indicating a tight link between CXCL16 and liver inflammatory injury (23).

However, the regulatory mechanism underlying abnormal CXCL16 expression in chronic hepatitis B-related hepatic inflammation remains largely unclear. Therefore, the present study aimed to investigate the association between CXCL16 gene promoter methylation and hepatic inflammation in patients with chronic hepatitis B, and to further elucidate the potential value of CXCL16 promoter methylation as a predictive indicator for hepatic inflammation.

# [Materials and methods]

### Participants

A total of 217 participants diagnosed with chronic hepatitis B (CHB) were prospectively collected from the Department of Hepatology, Qilu Hospital of Shandong University between August 2024 and August 2025. Meanwhile, 27 healthy individuals were enrolled to serve as healthy controls (HCs). All CHB patients had received long-term oral antiviral treatment with nucleos(t)ide analogues, including entecavir, tenofovir disoproxil fumarate, or combination therapy, for at least 1 consecutive year and remained on antiviral medication at study enrollment. The diagnostic criteria for CHB were based on the 2015 updated clinical practice guidelines released by the Asian Pacific Association for the Study of the Liver (APASL) (24). This study was performed in compliance with the ethical principles of the Declaration of Helsinki and approved by the Ethics Committee of Qilu Hospital of Shandong University. The inclusion criteria were as follows: (1) age≥18 years; (2) HBsAg seropositive status at 6 months or beyond. The exclusion criteria were as follows: (1) co-infection with other hepatitis viruses (hepatitis A, C, D, or E, or HIV); (2) comorbidities such as autoimmune hepatitis, alcoholic liver disease, non-alcoholic fatty liver disease, drug-induced liver injury, primary biliary cirrhosis, HCC, ACLF, or other types of cancer; (3) pregnant women; (4) patients with blood diseases, diabetes, or other potential underlying conditions.

### Observation indicators

Clinical characteristics and laboratory parameters assessed in this study included age, gender, serum biochemical markers (ALT, AST, TBil, Alb, and AFP), and HBV serological markers (HBsAg and HBeAg).

HBV serological parameters were analyzed using the Cobas 6000 analyzer system (Roche Diagnostics, Basel, Switzerland) at the Department of Laboratory Medicine, Qilu Hospital of Shandong University. Additionally, CXCL16 mRNA expression, CXCL16 promoter methylation level, and plasma CXCL16 concentration were quantitatively determined in all participants.

**Peripheral blood mononuclear cells (PBMCs) extraction**

All participants provided peripheral venous blood samples, which were anticoagulated using citrate.

Peripheral blood mononuclear cells (PBMCs) were purified using Ficoll-Paque Plus density gradient centrifugation (GE Healthcare, Uppsala, Sweden). Following isolation, the obtained cells were stored at −80 °C for subsequent molecular experiments (25).

**RNA extraction and RT-qPCR**

Total RNA was extracted from PBMCs using TRIzol reagent (Invitrogen).

Complementary DNA (cDNA) was then synthesized using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Fermentas, Vilnius, Lithuania) following the manufacturer's instructions. Subsequent quantitative real-time polymerase chain reaction (RT-qPCR) was performed on a CFX Connect Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA) using the synthesized cDNA as the amplification template. All reactions were carried out in a final volume of 10 μL according to the standard manufacturer's protocol under the following thermal cycling conditions: 95 °C for 30 s, followed by 45 cycles of 95 °C for 5 s, 60 °C for 30 s, and 72 °C for 30 s (26).

**Genomic DNA Extraction and Bisulfite Modification**

Genomic DNA was isolated from PBMCs using TRIzol Reagent (Invitrogen, CA, USA). Subsequent sodium bisulfite modification of genomic DNA was performed strictly in accordance with the manufacturer's protocol using the EZ DNA Methylation-Gold Kit (Zymoresearch, Orange, CA, USA).

**MethyLight Quantitative Methylation Analysis**

MethyLight real-time PCR assays for methylation detection were conducted with the EpiTect MethyLight PCR + ROX Vial Kit (QIAGEN, Hilden, Germany) (27). The promoter region of the CXCL16 gene (2000 bp upstream of the transcription start site, TSS) was delineated via the NCBI database (https://www.ncbi.nlm.nih.gov/), and the corresponding DNA sequence was processed for transformation using MethPrimer (https://www.urogene.org/methprimer/), which was also used to identify CpG islands within the target promoter region. Specific probes and primers for CXCL16 and β-actin (reference gene) were designed with Oligo7 software (OLIGO, Colorado Springs, CO, USA), and their sequences are detailed in Table 1. All MethyLight PCR reactions were carried out in a final volume of 10 μL following the kit's standard protocol with the following thermal cycling conditions: initial denaturation at 95 °C for 15 min, followed by 45 cycles of 95 °C for 15 s and annealing/extension at 60 °C for 1 min.

Methylation levels were quantified as the percentage of methylation reference value (PMR), calculated using the formula: PMR = 100% × 2−[ΔCt(target gene−control gene)Sample − ΔCt(target gene−control gene)Calibrator] (28).

### Enzyme-linked immunosorbent assay (ELISA)

The inflammatory parameters TNF-α, IL-1β, and CXCL16 were quantified using ELISA in plasma. The assays were performed by Lengton Bioscience Co, Shanghai, China, employing a competitive method for sample content detection. Absorbance was measured at 450 nm following the manufacturer's protocol (29).

### Statistical analysis

Quantitative variables were presented as medians with interquartile ranges (25th–75th percentiles), while categorical variables were summarized as counts and percentages (%). Comparisons of quantitative variables were performed using the Mann–Whitney U test and Kruskal–Wallis test, respectively. The chi-square test was applied for comparisons between categorical variables. Spearman correlation analysis was conducted to assess the association between CXCL16 promoter methylation levels and clinical parameters. The diagnostic performance of CXCL16 promoter methylation for hepatic inflammatory injury in patients with CHB was evaluated using the area under the receiver operating characteristic curve (AUC). All statistical analyses were carried out using IBM SPSS Statistics 27.0 (IBM Corporation, Somers, NY, USA) and GraphPad Prism 9.0 (GraphPad Software, San Diego, CA, USA). All tests were two-sided, and a P value < 0.05 was considered statistically significant.

# [Result]

### General characteristics of the study population

A total of 244 subjects were recruited in the present study, including 113 HBeAg-positive patients, 104 HBeAg-negative patients, and 27 healthy volunteers. The demographic characteristics and laboratory parameters of all participants are summarized in Table 2.

### Methylation status and mRNA expression of CXCL16 in HBeAg(+), HBeAg(-), and HCs groups

The methylation levels of the CXCL16 promoter in chronic hepatitis B (CHB) patients with different HBeAg serostatus were detected using the MethyLight method and compared with those in healthy individuals. The methylation levels in peripheral blood mononuclear cells (PBMCs) from CHB patients with different HBeAg serostatus and healthy controls (HC) are shown in Figure 1A. The results demonstrated that the methylation level of CXCL16 was significantly lower in HBeAg-positive CHB patients(median 3.04%, interquartile range 2.02-4.72%) than in HBeAg-negative CHB patients (median 6.10%, interquartile range 4.23–10.81%) and healthy controls (median 11.07%, interquartile range 8.98–12.44%).

As shown in Figure 1B, CXCL16 mRNA expression was significantly upregulated in HBeAg-positive CHB patients (median 17.65, interquartile range 14.24-22.11) compared with the HC group (median 1.54, interquartile range 0.63-2.59) and the HBeAg-negative CHB patient group (median 11.81, interquartile range 9.24-14.84), suggesting that CXCL16 expression was significantly increased in CHB patients and was associated with HBeAg status.

Furthermore, Spearman rank correlation analysis between the PMR values and mRNA expression levels of CXCL16 revealed a negative correlation (Figure 1C).

**Association between PMR values of CXCL16 and clinical features**

Spearman correlation analysis revealed that CXCL16 promoter methylation was significantly associated with HBsAg ($r = -0.1605$, $p = 0.0180$), HBeAg ($r = -0.3521$, $p < 0.0001$), ALT ($r = -0.1301$, $p = 0.0468$), AST ($r = -0.1429$, $p = 0.0288$), and Alb ($r = 0.1919$, $p = 0.0033$). In contrast, no significant correlations were observed between CXCL16 PMR values and HBV-DNA ($r = -0.0676$, $p = 0.5223$), AFP ($r = -0.0769$, $p = 0.3020$) or TBil ($r = -0.0291$, $p = 0.6582$). Correlation coefficients and corresponding p-values for all variables are presented in Table 3.

**Relationship between inflammation and CXCL16 promoter methylation level in CHB**

We hypothesized that CXCL16 methylation could act as a reliable biomarker for assessing oxidative stress in the progression of HBV infection. ELISA was used to detect inflammatory indicators in CHB patients with different HBeAg statuses and healthy subjects. The results verified that the levels of inflammatory factors such as TNF-α and IL-1β were obviously increased in HBeAg-positive CHB patients (Table 2 and Figure 2). Elevated serum CXCL16 levels were observed in HBeAg-positive CHB patients, as clearly demonstrated in Figure 2. More specifically, serum CXCL16 concentrations were significantly higher in this patient subgroup compared with HBeAg-negative CHB patients and healthy control individuals. Meanwhile, CXCL16 methylation levels in HBeAg-positive patients were markedly lower than those in HBeAg-negative patients and healthy controls. Correlation analysis was further performed to explore the relationship between CXCL16 promoter methylation and inflammatory indicators. As shown in Figure 3, CXCL16 promoter methylation level was significantly negatively correlated with TNF-α and IL-1β levels ($P<0.05$).

**CXCL16 Promoter Methylation Level as a Predictor of Inflammation in Chronic Hepatitis B Patients**

In this study, we found that the level of CXCL16 promoter methylation is also an important marker for the occurrence of inflammation in patients with HBV. When the critical values of TNF-α, IL-1β, CXCL16, and PMR were 50.335,29.487,947.785,4.109, respectively, the AUC were 0.635 (95%CI 0.549-0.721, $p=0.0033$), 0.646 (95%CI 0.557-0.735, $p=0.0014$), 0.659 (95%CI 0.571-0.748, $p<0.001$) and 0.749 (95%CI 0.670-0.829, $p<0.001$). Table 4; Fig. 4 show more details.

# [Discussion]

The present study investigated the association between CXCL16 promoter methylation and hepatic inflammation in patients with chronic hepatitis B (CHB). Our data demonstrated that CXCL16 promoter methylation was significantly reduced in CHB patients, especially in the HBeAg-positive subgroup, whereas CXCL16 mRNA and serum protein levels were markedly elevated. Meanwhile, CXCL16 promoter methylation was negatively correlated with CXCL16 expression, HBeAg status, ALT, AST, TNF-α, and IL-1β levels. ROC curve analysis further revealed that CXCL16 promoter methylation exhibited favorable diagnostic performance in evaluating hepatic inflammatory injury in CHB patients. Collectively, these findings indicate that CXCL16 promoter hypomethylation is closely associated with enhanced CXCL16 expression and aggravated hepatic inflammation in CHB, and may serve as a promising non-invasive biomarker for assessing CHB-related liver inflammation.

CXCL16 is a unique chemokine existing in both membrane-bound and secreted isoforms, acting as the only cognate ligand for CXCR6 (30-32). It is critically involved in immune cell recruitment, activation, and inflammatory signal transduction within the liver (33, 34). Under physiological conditions, CXCL16 maintains low-level expression to preserve hepatic immune homeostasis. In response to persistent HBV infection, however, CXCL16 is significantly upregulated, which promotes inflammatory cell infiltration and pro-inflammatory cytokine release, thereby exacerbating liver inflammation and injury. Consistent with previous studies, our results confirmed that serum CXCL16 levels were remarkably higher in CHB patients, particularly in HBeAg-positive individuals, supporting the pivotal role of CXCL16 in HBV-associated hepatic inflammation.

DNA methylation is a major epigenetic modification that negatively regulates gene transcription (35). In this study, we observed that CXCL16 promoter methylation was significantly lower in HBeAg-positive CHB patients than in HBeAg-negative patients and healthy controls. Moreover, CXCL16 promoter methylation was inversely correlated with CXCL16 mRNA expression, suggesting that hypomethylation of the CXCL16 promoter contributes to the transcriptional upregulation of CXCL16 in CHB. This finding provides a novel epigenetic mechanism underlying the aberrant expression of CXCL16 during HBV infection.

HBeAg is a key serological marker of HBV replication and is closely associated with the severity of hepatic inflammation in CHB patients (36, 37). Our correlation analysis showed that CXCL16 promoter methylation was negatively associated with HBeAg status, indicating that HBeAg-positive patients are more prone to CXCL16 promoter hypomethylation and subsequent CXCL16 overexpression. This phenomenon may be attributed to the immunomodulatory effect of HBeAg, which suppresses host innate and adaptive antiviral immunity, sustains a chronic hepatic inflammatory microenvironment, and further induces epigenetic alterations in chemokine genes such as CXCL16.

In addition, our study revealed that CXCL16 promoter methylation was negatively correlated with ALT and AST, two core serological indicators of hepatocellular damage. Furthermore, CXCL16 promoter methylation was inversely associated with the pro-inflammatory cytokines TNF-α and IL-1β (38). These results strongly suggest that CXCL16 promoter hypomethylation is closely linked to the intensity of

hepatic inflammation and hepatocyte injury in CHB. By upregulating CXCL16 expression, promoter hypomethylation may amplify the inflammatory cascade and accelerate the progression of liver injury.

ROC analysis demonstrated that CXCL16 promoter methylation achieved a relatively high AUC value in predicting hepatic inflammatory injury, supporting its potential as a non-invasive molecular indicator for evaluating inflammation severity in CHB patients. This biomarker may complement conventional liver function tests and help identify patients at risk of progressive inflammatory injury.

Several limitations of this study should be acknowledged. First, this is a single-center study with a limited sample size. Second, the precise molecular mechanism by which HBV regulates CXCL16 methylation remains to be further explored in basic experiments. Future multi-center, large-sample cohort studies and in-depth mechanistic investigations are warranted to verify the clinical value and regulatory network of CXCL16 promoter methylation in CHB.

# [Conclusion]

In conclusion, our study demonstrates that **CXCL16 promoter hypomethylation i**s significantly associated with increased CXCL16 expression and elevated levels of inflammatory cytokines including TNF-α and IL-1β in patients with chronic hepatitis B. CXCL16 promoter methylation is negatively correlated with HBeAg status, ALT, and AST levels, and shows good diagnostic value for evaluating hepatic inflammatory injury. These findings indicate that CXCL16 promoter methylation may serve as a **novel non-invasive molecular biomarker** for assessing liver inflammation in CHB patients. This work enriches the epigenetic regulatory mechanism underlying HBV-related hepatic inflammation and provides a potential target for the clinical evaluation and targeted intervention of chronic hepatitis B. Further multi-center, large-sample clinical studies and in-depth mechanistic experiments are needed to verify these results and clarify the precise regulatory pathway of CXCL16 in HBV infection.

# Abbreviations

HBV Hepatitis B virus

CXCL16 C-X-C motif chemokine ligand 16

HBeAg Hepatitis B e Antigen

HBsAg Hepatitis B surface antigen

CHB Chronic hepatitis B

HCs Healthy controls

HCC Hepatocellular carcinoma

PMR The percentage of methylation reference

ALT Alanine aminotransferase

AST Aspartate aminotransferase

TBIL Total bilirubin

ALB Albumin

AFP Alpha-fetoprotein

TNF-α Tumor Necrosis Factor-alpha

IL-1β Interleukin-1 beta

# Declarations


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### Author contributions

Kai Wang designed the study and carried it out. Zhezhe Tian, Miaomiao Xu, Hongfei Li, Yunxin Yao, and YuChen Fan collect data. Zhezhe Tian conducted the statistical analysis and drafted the manuscript. All the authors read and approved the manuscript.

### Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Medical Ethical Committee of Qilu Hospital of Shandong University. (#KYLL-202306-021-1).

### Availability of data and materials

No datasets were generated or analysed during the current study.

### Competing interests

The authors declare no competing interests.


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### Consent for publication

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

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# Tables

**Table1.** Sequences of used primers and probes

| Gene | Forward primer sequence (5’-3’) | Primer/probe sequence(5’-3’) |
|---|---|---|
| **RT-qPCR** | | |
| CXCL16 | CCGGAAACACCTGAGAGCTT | AAAGGAGCTGGAACCTCGTG |
| β-actin | CATGTACGTTGCTATCCAGGC | CTCCTTAATGTCACGCACGAT |
| **Methylight** | | |
| CXCL16 | TTATCGGGAACGGTTTGGTG | CGCCACGCAAATCCGA |
| ACTB | TGGTGATGGAGGAGGTTTAGTAAGT | AACCAATAAAACCTACTCCTCCCTTAAA |
| **Probe oligo sequence** | | |
| CXCL16 | TTGGATGCGTTTGTGGG | |
| ACTB | ACCACCACCCAACACACAATAACAAACACA | |

**Table2.** Baseline Characteristics of Participants

| Variable | HBeAg(+)(n=113) | HBeAg(-) (n=104) | HCs(n=27) | P value |
|---|---|---|---|---|
| Age (years) | 39.00 (35.00,44.50) | 40.00 (36.00,48.75) | 39.000 (35.00,45.00) | 0.281[a] |
| Male, n(%) | 63 (55.8%) | 66 (63.5%) | 12 (44.4%) | 0.171[c] |
| ALT (U/L) | 24.00 (17.00,39.00) | 22.50 (15.25,29.00) | 16.00 (10.00,24.00) | 0.001[a] |
| AST (U/L) | 22.00 (18.50,30.50) | 21.00 (17.00,25.00) | 17.00 (14.00,22.00) | 0.002[a] |
| TBIL (μmol/L) | 11.00 (8.20,14.20) | 12.70 (8.73,15.88) | 8.20 (4.90,10.90) | <0.001[a] |
| ALB (g/L) | 46.80 (45.13,48.30) | 47.50 (45.73,48.70) | 47.20 (44.90,49.80) | 0.202[a] |
| HBsAg (IU/ml) | 4466.42 (1617.38,17165.18) | 1777.43 (426.33,5809.76) | NA | <0.001[b] |
| HBeAg (IU/ml) | 42.09 (4.18,879.90) | 0.41 (0.38,0.45) | NA | <0.001[b] |
| Log10[HBV-DNA] | 3.01 (2.42,4.95) | 2.32 (2.11,3.03) | NA | 0.004[b] |
| AFP (ng/ml) | 2.75 (2.05,3.96) | 2.68 (1.79,3.96) | 2.65 (2.51,3.10) | 0.719[a] |

a Kruskal–Wallis H test. b Mann–Whitney U test. c Chi-square test

**Table3.** Correlation between PMR% and liver function parameter

| Parameter | PMR% | |
|---|---|---|
| | *r* value | *P* value |
| **HBV DNA** | -0.0676 | 0.5223 |
| **HBsAg** | -0.1605 | 0.0180* |
| **HBeAg** | -0.3521 | <0.0001 |
| **AFP** | -0.0769 | 0.3020 |
| **ALT** | -0.1301 | 0.0468* |
| **AST** | -0.1429 | 0.0288 |
| **TBil** | -0.0291 | 0.6582 |
| **Alb** | 0.1919 | 0.0033** |

**Table 4** AUC and cut-off values of prognostic variables

| ROC | AUC | | Sensitivity | Specificity | Cut-off | *P* value |
|---|---|---|---|---|---|---|
| | Area | 95%CI | | | | |
| **TNF-α** | 0.635 | 0.549-0.721 | 0.375 | 0.850 | 50.335 | 0.0033 |
| **IL-1β** | 0.646 | 0.557-0.735 | 0.650 | 0.688 | 29.487 | 0.0014 |
| **CXCL16** | 0.659 | 0.571-0.748 | 0.725 | 0.725 | 947.785 | 0.0004 |
| **PMR** | 0.749 | 0.670-0.829 | 0.762 | 0.762 | 4.109 | 0.0001 |

# Figures

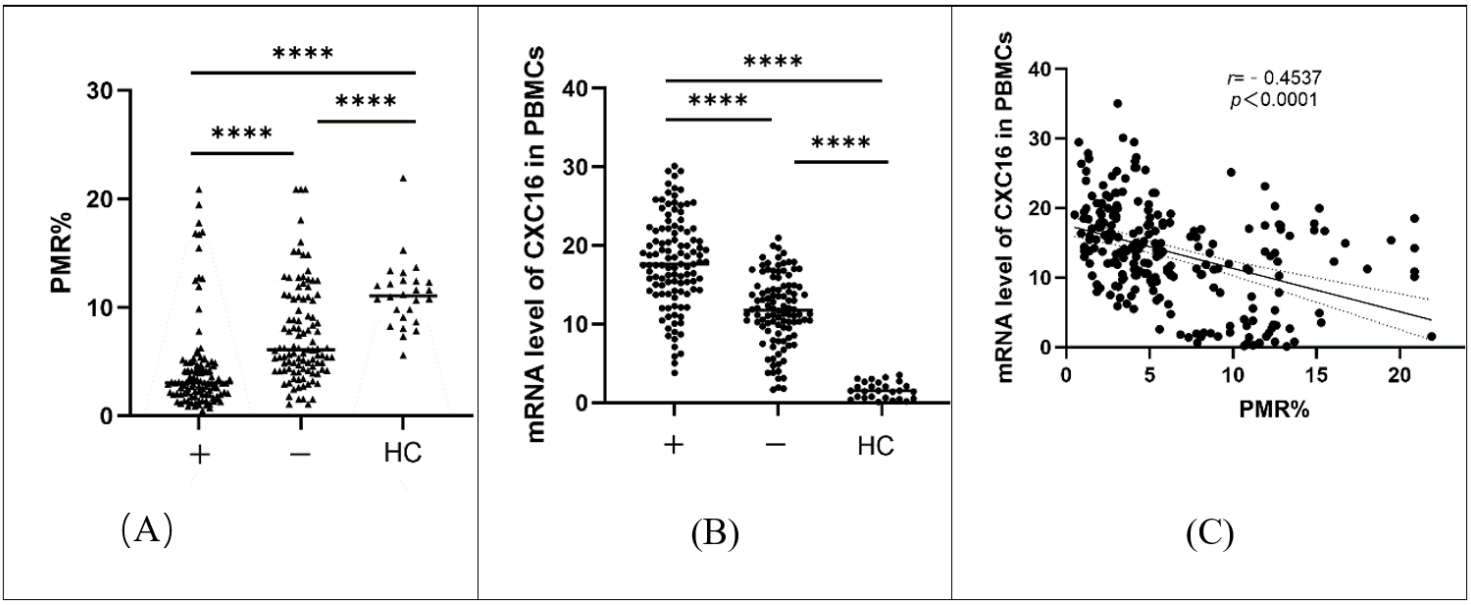



**Figure 1**

CXCL16 promoter methylation levels, CXCL16 mRNA levels, and their correlation in HBeAg-positive patients, HBeAg-negative patients and healthy controls (HCs).

(**A**)The CXCL16 methylation level was significantly lower in the HBeAg(+) group than in HBeAg(-) ($p$<0.0001) and HCs group ($p$<0.0001). (**B**) CXCL16 mRNA level was significantly higher in the HBeAg(+) group than in HBeAg(-) ($p$<0.0001) and HCs group ($p$<0.0001). (**C**) Spearman rank correlation analysis between the PMR values and mRNA expression levels of CXCL16 revealed a negative correlation Relationships between CXCL16 methylation level and mRNA level in PBMCs. ****$p$<0.0001,***$p$<0.001, **$p$<0.01, *$p$<0.05.

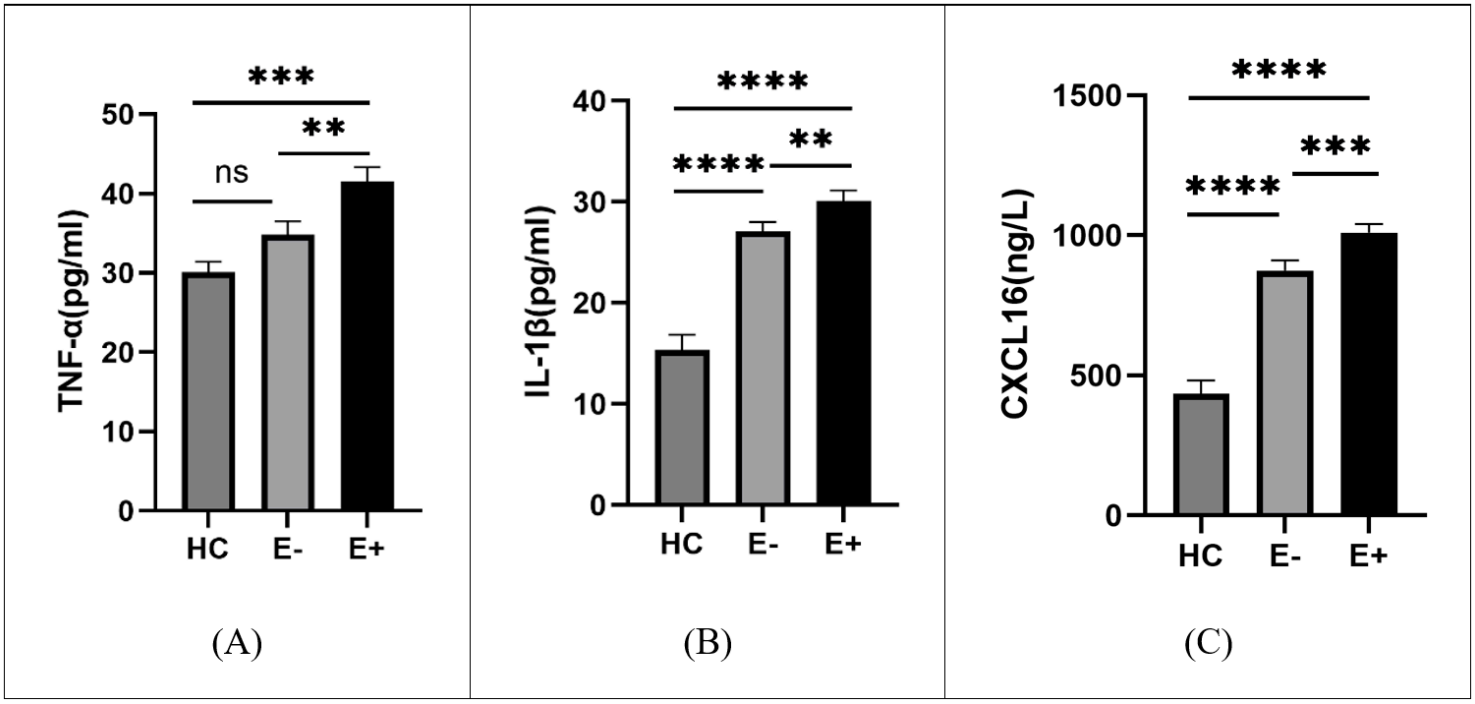


**Figure 2**

Serum CXCL16, TNF-α, and IL-1β levels in HBeAg-positive patients, HBeAg-negative patients and healthy controls (HCs). (**A**) Serum TNF-α levels were significantly higher in patients with HBeAg(+) as compared with HBeAg(-) (*P*=0.0033) and healthy controls (*P*=0.0002). (**B**) Serum IL-1β levels were significantly higher in patients with HBeAg(+) as compared with HBeAg(-) (*P*=0.0014) and healthy controls (*P*<0.0001). (**C**) Serum CXCL16 levels were significantly lower in patients with HBeAg(+) as compared with HBeAg(-) (*P*=0.0004) and healthy controls (*P*<0.0001). Statistical differences were analyzed using a two-tailed Mann-Whitney U test.****p < 0.0001,****p*<0.001, **p*<0.01, *p*<0.05

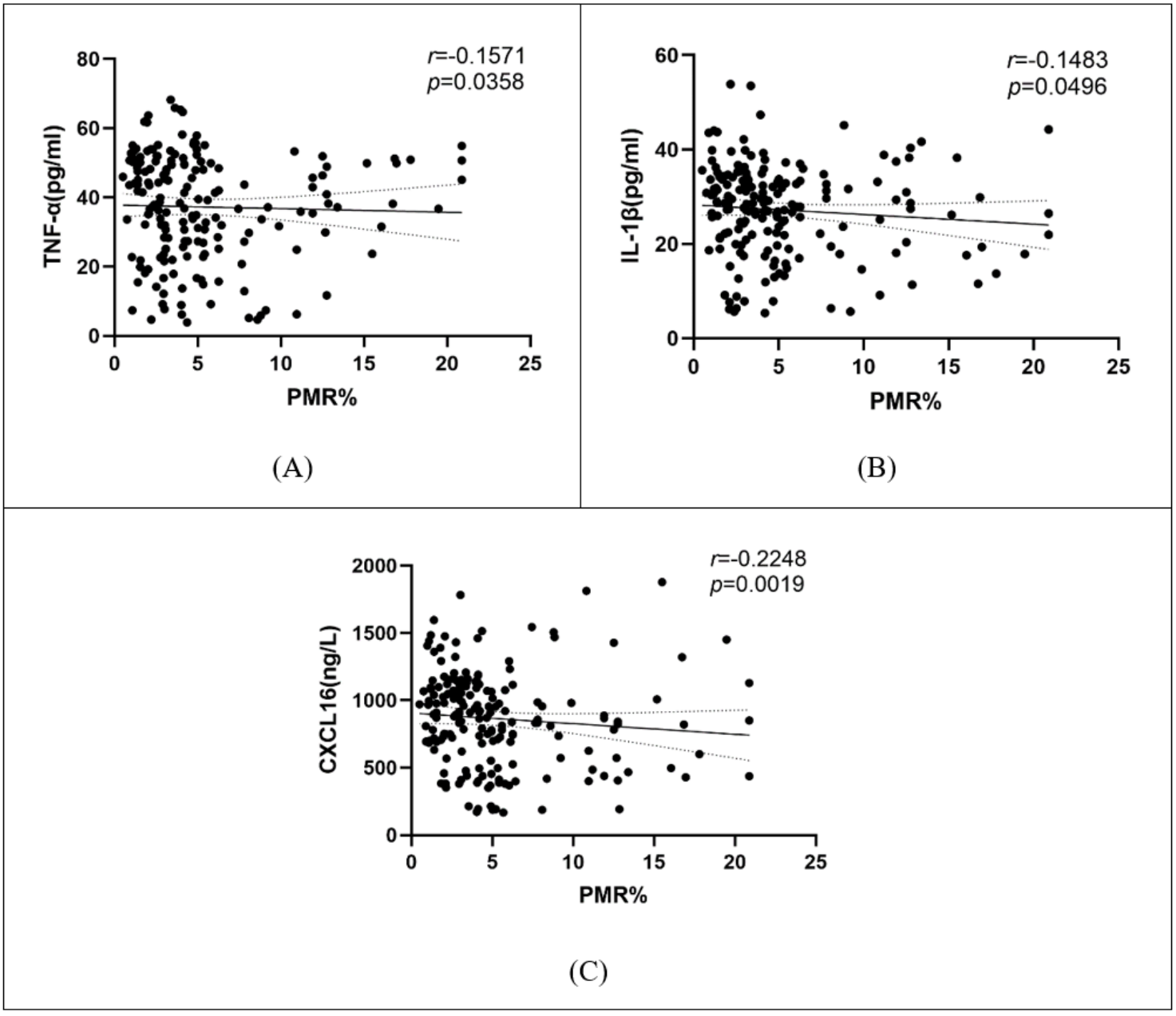



**Figure 3**

Relationships between CXCL16 methylation level, Serum CXCL16, TNF-α and IL-1β.

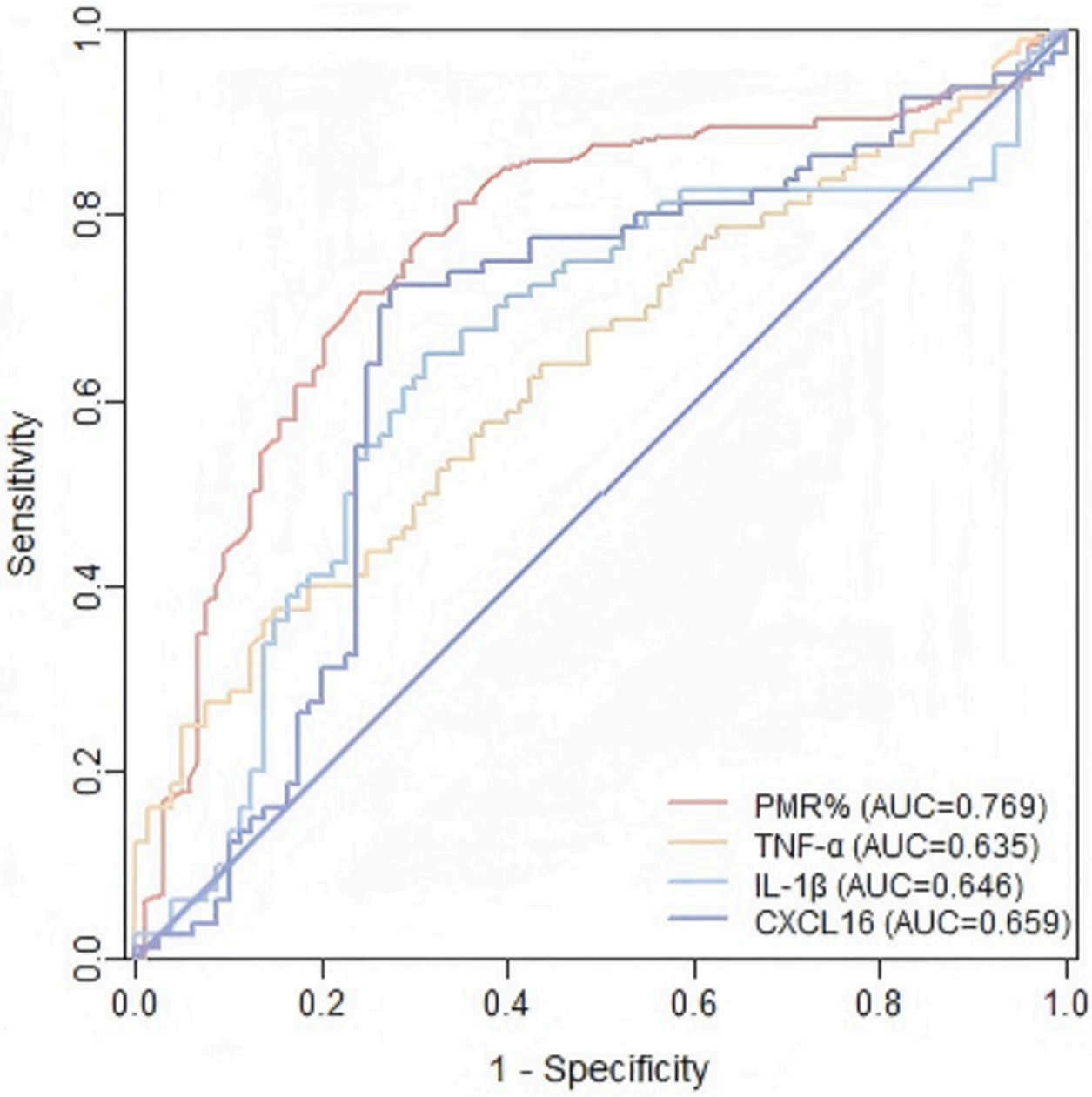



**Figure 4**

The diagnostic value of CXCL16 promoter methylation levels in HBeAg(+)CHB. The ROC of CXCL16 promoter methylation level was better than serum CXCL16,TNF-α,and IL-1βin CHB patients.