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The value of promoter methylation of fibroblast factor 21 (FGF21) in predicting the course of chronic hepatitis B and the occurrence of oxidative stress

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Abstract

Background Oxidative stress plays a crucial role in the pathogenesis of HBV. This study aimed to investigate the value of fibroblast growth factor 21 (FGF21) promoter methylation in the occurrence and development of chronic hepatitis B (CHB) oxidative stress.

Methods A total of 241 participants including 221 patients with CHB and 20 healthy controls (HCs) were recruited. Methylation level of FGF21 promoter in peripheral blood mononuclear cells was quantitatively determined. Enzyme-linked immunosorbent assay was used to assess oxidative stress in CHB patients.

Results Our study shows that the FGF21 methylation level was significantly lower in HBeAg-positive CHB patients compared to HBeAg-negative CHB patients and HCs ($P < 0.0001$). The oxidative stress of HBeAg-positive CHB patients was more severe. Further correlation analysis showed that there was a significant correlation between the methylation level of FGF21 promoter and the occurrence of oxidative stress in CHB patients. In addition, assessment based on FGF21 promoter methylation level proved effective for predicting oxidative stress occurrence and disease progression among CHB patients.

Conclusion FGF21 promoter methylation level is an important marker for predicting oxidative stress and disease progression in patients with CHB.

Keywords FGF21, HBV, Oxidative stress, Methylation

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Introduction

Hepatitis B virus (HBV) infection is a significant global public health concern, with early HBV infection often resulting in lifelong chronic infection and viral carrier status. Late-stage HBV infection typically leads to either acute, self-limiting diseases or liver failure due to viral clearance, posing a substantial threat to the health of millions of patients, particularly in the Asia-Pacific region and China [1]. HBeAg, as a non-structural protein directly synthesized and secreted from hepatocytes in the endoplasmic reticulum of HBV-infected patients, serves as a reference standard for reflecting the level of HBV replication [2]. For chronic hepatitis B (CHB) patients with HBeAg positivity, achieving Hepatitis B e Antigen (HBeAg) seroconversion is currently the treatment goal. This not only signifies partial immunological control but also represents a prerequisite for clinical cure of CHB [3]. HBV can disrupt protein folding in host cells, impair lipid metabolism, and induce endoplasmic reticulum stress that triggers oxidative stress [4–6]. Oxidative stress forms the backdrop for viral and alcoholic liver diseases' development and participates in liver fibrosis formation. The pathogenesis involves hepatocytes, stellate cells, endothelial cells, and Kupffer cells; it encompasses ischemia/reperfusion events along with necrosis and apoptosis processes [7–9]. Prolonged oxidative stress may lead to chronic inflammation in the liver while inducing hepatocyte injury—a pivotal factor influencing injury—repair equilibrium crucial for maintaining homeostasis. In the context of HBV infection's progression, oxidative stress plays an essential role in counteracting therapeutic efficacy as well as downregulating surface antigen and HBeAg levels. Fibroblast growth factor 21 (FGF21) is an atypical member of the fibroblast growth factor signalling system, primarily secreted by the liver. Serum FGF21 demonstrates superior sensitivity for detecting liver ischemia/reperfusion injury compared to currently utilized biomarkers. Its involvement has been partially elucidated in diabetes, cardiovascular diseases, and metabolic-related liver disorders. Notably, a study [10] reported that elevated FGF21 levels correlate with poor survival outcomes in patients with hepatocellular carcinoma (HCC), suggesting that increased serum FGF21 may serve as a prognostic indicator for HCC. Studies [11–13] have also established that FGF21 acts as a negative regulator of bile acid synthesis within the liver and is modulated by PPARα. Furthermore, previous investigation [14] has identified FGF21 as a target gene of ATF4 and CHOP, these transcription factors mediate endoplasmic reticulum stress-induced expression of FGF21 at both transcriptional and mRNA levels. In models lacking FGF21, there was a significant increase in endoplasmic reticulum stress alongside heightened rates of cellular apoptosis. Additionally, FGF21 regulates endoplasmic reticulum stress while diminishing lipogenesis and inflammatory cytokine production, thereby enhancing lipid consumption and degradation processes [15]. Collectively, these findings underscore the pivotal role of FGF21 in the pathogenesis of various metabolic disorders and hepatic diseases; thus its expression level may be crucial during HBV infection and oxidative stress responses. For CHB patients with HBV infection, early detection and antiviral treatment are crucial for slowing down liver fibrosis progression and improving survival rate. In this study, we mainly used high-sensitivity and specific quantitative techniques to analyze DNA methylation to detect the role of FGF21 methylation in the response to HBV infection and the oxidative stress process and to elucidate the potential clinical value of FGF21 methylation level as a biomarker for oxidative stress and HBeAg seroconversion during the progression of CHB.

Materials and methods

Participants

This prospective study enrolled 221 patients with CHB who were admitted to the Department of Hepatology, Qilu Hospital of Shandong University, from August 2023 to August 2024. Additionally, 20 healthy controls (HCs) were recruited. All CHB patients were given antiviral therapy with nucleotide analogues by mouth Entecavir, Tenofovir disoproxil fumarate or both were treated continuously for more than 1 year(Those patients are still receiving the antiviral treatment). The inclusion criteria for CHB patients were determined in accordance with the updated CHB management practice guidelines of the Asian Pacific Association for the Study of the Liver (APASL) in 2015 [16]. This study was conducted following the relevant provisions of the Helsinki Declaration and approved by the Ethics Committee of Qilu Hospital of Shandong University. The inclusion criteria were as follows: (1) age≥18 years; (2) ALT≥40 U/L; (3) HBsAg seropositive status at 6 months or beyond. The exclusion criteria were as follows: (1) co-infection with other hepatitis viruses (hepatitis A, B, 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

The observation indicators included in this study included age, gender, serum biochemical parameters (ALT, AST, TBil, Alb, and AFP), and HBV serological parameters (including HBsAg, HBeAg, and HBeAb). The HBV serological parameters were measured using the Cobas 6000 analyzer series produced by Roche

Diagnostics (Basel, Switzerland) and detected by the Laboratory of Qilu Hospital of Shandong University. In addition, we measured the FGF21 mRNA level, the FGF21 promoter methylation level, the plasma oxidative stress level, and the plasma FGF21 level.

Peripheral blood mononuclear cells (PBMCs) extraction

All subjects' peripheral blood was anticoagulated with citrate. After centrifugation on Ficoll-Paque Plus (GE Healthcare, Uppsala, Sweden) gradient density centrifuge, mononuclear cells were collected. They were then placed in a -80 °C freezer for storage until use.

RNA extraction and RT-qPCR

Use TRIzol Reagent (Invitrogen) to extract total RNA from PBMCs in this study. Subsequently, the cDNA was synthesized according to the instructions provided by RevertAid First Strand Thermo Fisher Scientific cDNA Synthesis Kit (Fermentas, Vilnius, Lithuania). The resulting cDNA served as a template for reverse-transcriptase polymerase chain reaction (RT-qPCR), which was performed using the CFX Connect real-time PCR system (Bio-Rad Laboratories, Hercules, CA) for real-time detection. We use the standard protocol provided by the manufacturer with a total volume of 10 µl. The reaction procedure is as follows: 95 °C for 30s, followed by 45 cycles of 95 °C for 5s, 60 °C for 30s and 72 °C for 30s [17].

DNA extraction and quantitative methylation-specific PCR (MethyLight) using TaqMan probes

The genomic DNA was extracted from PBMC using TRIzol Reagent (Invitrogen). For DNA bisulfite modification, the EZ DNA Methylation-Gold kit (Zymoresearch, Orange, CA, USA) was employed. MethyLight analysis was conducted using the EpiTect MethyLight PCR+ROX Vial Kit (QIAGEN, Hilden, Germany). We used the website (https://www.ncbi.nlm.nih.gov/) to delineate the promoter of FGF21 and another website (https://www.urogene.org/methprimer/) for sequence transformation. Then, the Oligo7 (OLIGO 1267 Vondelpark ColoradoSprings, CO80,907, USA) was used for the sequence design of probes and primers. We selected the upstream 2,000 bp region of its transcription start site (TSS) as the promoter region. Then, we found one CpG island through the website (https://www.urogene.org/methprimer/) [18]. So, the primers and probes were designed. The FGF21 and B-actin gene-specific primers and probe sequences are listed in Table 1. We used a total volume of 10 µl following the standard protocol provided by the manufacturer: 95 °C for 15 min, followed by 45 cycles of 95 °C for 15s and 60 °C for 1 min [17]. The percentage of methylation reference value (PMR) represents the MethyLight data. PMR = 100% x $2^{-[\Delta Ct(\text{target gene}-\text{control gene})\ \text{Sample}-\ \Delta Ct(\text{target gene}-\text{control gene})]}$ [19].

Table 1 Sequences of used primers and probes

Gene	Forward primer sequence (5′-3′)	Primer/probe sequence (5′ – 3′)
RT-qPCR		
FGF21	CTGCAGCTGAAAGCCTTGAAGC	GTATCCGTCCTCAA GAAGCAGC
ACTB	ATGGGTCAGAAGGATTCCTATGTG	CTTCATGAGGTAGT CAGTCAGGTC
Methylight		
FGF21	TTATTAAGACGTAGAGATCGGTAGT	TCACGTAACTTACT TAACCTTATCAAT
ACTB	TGGTGATGGAGGAGGTTTAGTAAGT	AACCAATAAAACCT ACTCCTCCCTTAAA
Probe oligo sequence		
FGF21	AACGACTCACCCTCCTTATCCTACCC	
ACTB	ACCACCACCCAACACACAATAACAAACACA	

Enzyme-linked immunosorbent assay (ELISA)

The oxidative parameters 8-hydroxy-2'-deoxyguanosine(8-OHdG) and the antioxidant parameters superoxide dismutase (SOD), catalase (CAT), and FGF21 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.

Statistical analysis

Quantitative variables are expressed as median (centile 25; centile 75). Categorical variables were expressed as numbers (%). Mann–Whitney U test and Kruskal-Wallis Test were used to compare the quantitative variables. The chi-square test was used to compare the categorical variables. Spearman correlation is employed to analyze the relationship between FGF21 promoter methylation status and clinical data. Furthermore, the diagnostic value of FGF21 promoter methylation status for oxidative stress injury response in HBV patients is evaluated using the area under the receiver operating characteristic curve (AUC). All statistical analyses were performed using SPSS version 27.0 software (IBM Corporation, Somers, NY, USA) and GraphPad Prism 9.0 (San Diego, CA, USA). All statistical analyses were 2-sided, and P value < 0.05 was considered statistically significant.

Results

General characteristics of the study population

A total of 241 participants were enrolled in this study, comprising 115 HBeAg seropositive patients, 106 HBeAg(-) seronegative patients, and 20 healthy individuals. The demographic and laboratory data of the participants are detailed in Table 2.

Table 2 General clinical characteristics of the patients

Parameter	HCs(20)	HBeAge(-)(106)	HBeAg(+) (115)	*P* value
Age(years)	33.50(25.00,43.50)	42.00(35.00,49.25)	39.00(35.00,47.00)	0.304[b]
Male, n (%)	5(25.00)	69(65.00)	76(66)	0.002[c]
Treatment, n (%)	NA			
Entecavir		65(61.32)	67(58.26)	0.862[c]
Tenofovir disoproxil fumarate		35(33.02)	43(37.39)	0.365[c]
Other/combination		6(5.66)	5(4.35)	0.763[c]
HBsAg(IU/ml)	NA	1537.85(383.43,3594.76)	4708.58(1705.74,17306.25)	< 0.001[a]
HBeAg(IU/ml)	NA	0.41(0.36,0.63)	26.29(2.77,800.28)	< 0.001[a]
HBeAb(IU/ml)	NA	0.02(0.02,0.03)	3.70(1.66,42.42)	< 0.001[a]
log10[HBV-DNA]	NA	3.26(2.67,3.78)	4.15(3.36,5.64)	< 0.001[a]
AFP(ng/ml)	3.08(2.65,3.11)	2.53(1.87,3.54)	2.78(2.03,3.92)	0.148[b]
ALT(U/L)	16.00(12.00,23.25)	47.00(44.00,51.00)	51.00(45.00,60.00)	0.001[b]
AST(U/L)	18.50(14.75,20.25)	39.00(35.00,,45.00)	44.00(36.00,52.00)	< 0.001[b]
TBil(umol/L)	10.40(6.00,12.90)	11.30(8.40,15.23)	11.30(8.10,15.50)	0.893[b]
Alb(g/L)	48.90(47.10, 50.20)	48.20(46.00,49.95)	47.90(46.10,50.30)	0.999[b]
mRNA	1.62(0.38,7.47)	3.64(1.94,11.48)	19.30(4.99,40.70)	< 0.001[b]
PMR(%)	17.69(16.56,20.07)	16.68(14.50,18.42)	15.02(13.41,16.60)	< 0.001[b]
FGF21(ng/ml)	819.04(533.98,1202.50)	938.97(643.39,1229.30)	1236.48(930.53,1802.12)	< 0.001[b]
8-OHdG(ng/ml)	2.26(0.83,4.57)	5.98(3.39,11.35)	10.59(5.62,16.80)	< 0.001[b]
CAT(ng/ml)	26.88(24.48,34.24)	20.19(13.81,27.83)	17.77(15.10,20.40)	0.009[b]
SOD(ng/ml)	12.74(9.80,14.42)	10.26(8.37,12.30)	8.87(7.43,10.21)	0.001[b]

Quantitative variables were expressed as medians (25th, 75th)

Qualitative variables were expressed as number (percentage)

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

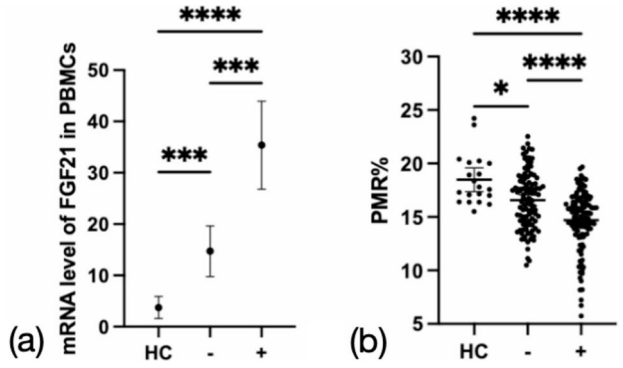

Fig. 1 FGF21 mRNA level and FGF21 promoter methylation level in patients with HBeAg(+), HBeAg(-) and HCs. (**a**) FGF21 mRNA level was significantly higher in the HBeAg(+) group than in HBeAg(-) (, $p=0.0002$) and HCs group ($p<0.0001$). (**b**)The FGF21 methylation level was significantly lower in the HBeAg(+) group than in HBeAg(-) ($p<0.0001$) and HCs group ($p<0.0001$). ****$p<0.0001$,***$p<0.001$, **$p<0.01$, *$p<0.05$

Methylation status of the FGF21 promoter in HBeAg(+), HBeAg(-), and HCs groups

We assessed the methylation levels of the FGF21 promoter in CHB patients with different HBeAg serostatus and compared them with those of healthy individuals using PMR analysis. The methylation levels in PBMCs from different HBeAg serostatus groups among CHB patients and healthy controls are depicted in Fig. 1. As illustrated, the methylation level of FGF21 was significantly lower in HBeAg(+) CHB patients than that in HBeAg(-) CHB patients (median 15.02%, interquartile range 13.41–16.60%, $P<0.0001$) as well as HCs (median 17.69%, interquartile range 16.56–20.07%, $P<0.0001$). Additionally, the methylation level of FGF21 promoter was notably reduced in CHB patients compared to HCs ($P<0.05$).

Relationship between FGF21 promoter methylation level and HBeAg status and liver function

Spearman correlation analysis showed that the promoter methylation level of FGF21 was significantly correlated with HBeAg (r = -0.225, $p=0.001$), HBeAb(r = -0.246, $p<0.001$), ALT (r = -0.149, $p=0.021$) and AST (r = -0.210, $p=0.001$). However, there was no significant correlation between PMR and HBV-DNA (r = -0.208, $p=0.077$), HBsAg (r = -0.046, $p=0.496$), the AFP ($r=0.015$, $p=0.834$), TBil (r = -0.059, $p=0.372$) and Alb (r = -0.028, $p=0.670$). The correlation coefficient of each parameter is expressed as an *R/P* value. The correlation analysis is shown in Table 3.

Relationship between oxidative stress and FGF21 promoter methylation level in CHB

We hypothesized whether FGF21 methylation could serve as a valuable biomarker for detecting oxidative stress in HBV infection. We employed ELISA to measure

Table 3 Correlation between PMR% and liver function parameter

Parameter	PMR%	
	***r* value**	***P* value**
HBV-DNA	-0.208	0.077
HBsAg	-0.046	0.496
HBeAg	-0.225	0.001
HBeAb	-0.246	< 0.001
AFP	0.015	0.834
ALT	-0.149	0.021*
AST	-0.210	0.001**
TBil	-0.059	0.372
Alb	-0.028	0.670

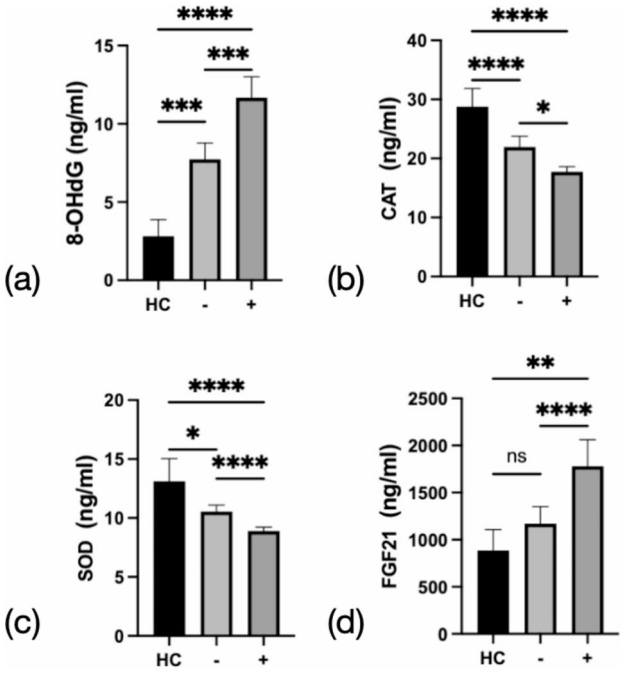

Fig. 2 Serum FGF21, 8-OHdG, CAT and SOD levels in e(+)CHB, e(-)CHB and HCs patients. (**a**) Serum 8-OHdG levels were significantly higher in patients with HBeAg(+) as compared with HBeAg(-) ($P=0.0002$) and healthy controls ($P<0.0001$). (**b**) Serum CAT levels were significantly lower in patients with HBeAg(+) as compared with HBeAg(-) ($P=0.0150$) and healthy controls ($P<0.0001$). (**c**) Serum SOD levels were significantly lower in patients with HBeAg(+) as compared with HBeAg(-) ($P<0.0001$) and healthy controls ($P<0.0001$). (**d**) Serum FGF21 levels were significantly higher in patients with HBeAg(+) as compared with HBeAg(-) ($P<0.0001$) and healthy controls ($P=0.0011$). Horizontal lines represent mean values for each group. Statistical differences were analyzed using a two-tailed Mann-Whitney U test.****p \ < 0.0001,***$p<0.001$, **$p<0.01$, *$p<0.05$

the levels of oxidative stress in CHB patients with different HBeAg statuses and HCs. Our findings revealed that CHB patients with HBeAg(+) exhibited elevated levels of oxidative stress and reduced antioxidant capacity, as depicted in Table 2; Fig. 2. Additionally, we observed higher serum FGF21 and mRNA levels in CHB patients with HBeAg(+) compared to those with HBeAg(-) ($P<0.001$). Furthermore, the methylation level of FGF21 was lower in HBeAg(+) patients than in HBeAg(-) patients ($P<0.0001$), as illustrated in Fig. 2. Subsequently, we performed correlation analysis between the methylation level of the FGF21 promoter and markers of oxidative stress and antioxidant status. Interestingly, we found that the methylation level of FGF21 promoter was negatively correlated with the oxidation index 8-OHdG ($P<0.05$), and it was positively correlated with CAT and SOD antioxidant indexes, as shown in Fig. 3.

The level of FGF21 in serum of CHB patients with HBeAg(+) is elevated

The serum level of FGF21 is elevated in CHB patients with HBeAg(+) as depicted in Fig. 2. Specifically, the level of FGF21 in the serum of CHB patients with HBeAg(+) is significantly higher than that of CHB patients with HBeAg(-) and HCs. No significant difference was observed in the level of FGF21 between CHB patients with HBeAg(-) and HCs. Furthermore, Spearman correlation analysis revealed a significant negative correlation between the methylation level of the FGF21 promoter and both FGF21 mRNA expression level and serum FGF21 expression level, as illustrated in Fig. 4.

Predicting oxidative stress in CHB patients by FGF21 promoter methylation level

In this study, we found that the level of FGF21 promoter methylation is also an important marker for the occurrence of oxidative stress in patients with HBV. When the critical values of 8-OHdG, CAT, SOD, FGF21, and PMR were 7.042, 23.982, 10.972, 1039.105, and 16.983, respectively, the AUC were 0.699 (95%CI 0.632–0.765, $p<0.0001$), 0.658 (95%CI 0.587–0.729, $p<0.0001$), 0.699 (95%CI 0.634–0.765, $p<0.0001$), 0.689 (95%CI 0.623–0.756, $p<0.0001$), and 0.706 (95%CI 0.641–0.771, $p<0.0001$). Table 4; Fig. 5 show more details.

Discussion

Oxidative stress can lead to the generation of reactive oxygen species (ROS), which can damage cellular mitochondria and disrupt the balance between free radical production and elimination. The regulation of free radicals and antioxidants within cells is important to prevent oxidative stress and cellular injury [20]. Recently, one study [21] has shown that patients with CHB exhibit increased ROS production within liver tissue leading to disruptions in various aspects of liver function. This is due to the increased production of free radicals and the weakening of antioxidant defenses. Oxidative stress is associated with multiple forms of CLD including viral hepatitis, necrosis, fibrosis cirrhosis, and HCC [22]. Patients infected with HBV exhibit varying degrees of oxidative damage, and the research has revealed accumulation of 8-OHdG in the livers of CHB patients [23, 24].

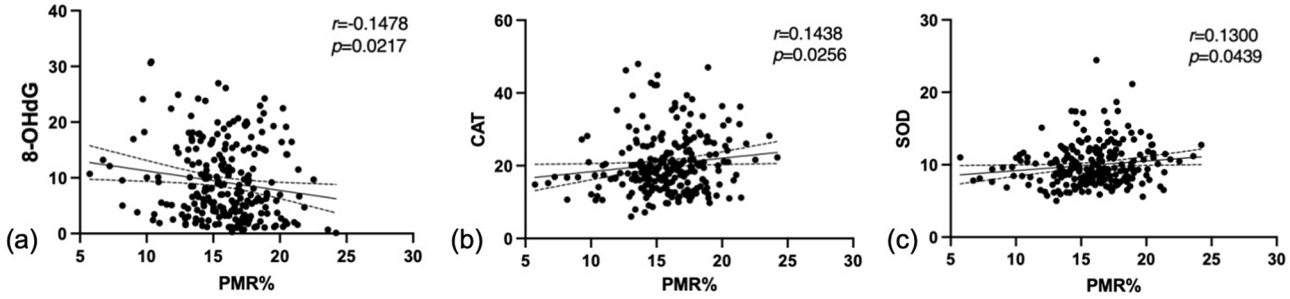

Fig. 3 Relationships between FGF21 methylation level, Serum 8-OHdG, CAT and SOD

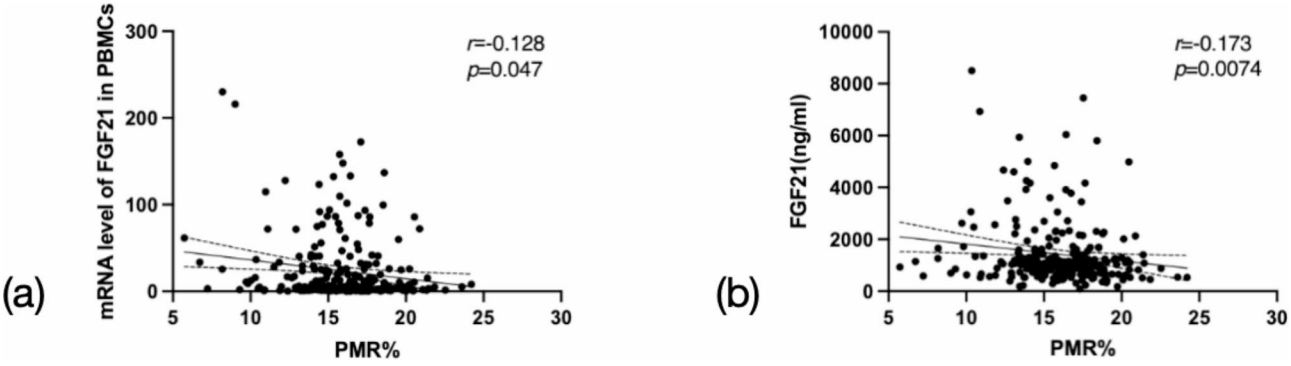

Fig. 4 Relationships between FGF21methylation level and mRNA level in PBMCs, and FGF21 expressive in serum

Table 4 AUC and cut-off values of prognostic variables

ROC	AUC		Sensitivity	Specificity	Cut-off	*P* value
	Area	95%CI				
8-OHdG	0.699	0.632–0.765	0.702	0.672	7.042	< 0.0001
CAT	0.658	0.587–0.729	0.921	0.432	23.982	< 0.0001
SOD	0.699	0.634–0.765	0.86	0.456	10.972	< 0.0001
FGF21	0.689	0.623–0.756	0.675	0.608	1039.105	< 0.0001
PMR	0.706	0.641–0.771	0.842	0.528	16.983	< 0.0001

The level of HBV-DNA replication activity in hepatocytes serves as an indicator for HBV protein replication activity and correlates with an increased risk of liver disease progression [25, 26]. Chen et al. [27] have demonstrated a close relationship between HBV-DNA load and serum HBeAg levels in CHB patients. However, previous study [28] has reported a significant disparity in the detection of HBeAg and HBV-DNA load. It is crucial to comprehend the dynamic course of HBV infection, explore markers for disease progression and serological transitions, as well as investigate the precise mechanisms underlying HBV infection, liver damage, and repair. These are essential to mitigate the occurrence of HBV-related complications such as cirrhosis, liver failure, and HCC.

Currently, both domestically and internationally, the primary approach for managing hepatitis B virus involves the utilization of antiviral medications such as NUCs in combination with interferon to suppress viral replication. However, due to the persistent presence of covalently closed circular DNA (cccDNA) among patients with CHB, complete eradication remains unattainable, leading to potential virus reactivation after treatment cessation [29]. Throughout the course of antiviral therapy, assessing HBsAg, HBeAg, and seroconversion in blood serum is crucial for predicting patient prognosis. While hepatitis B markers like HBeAg, HBsAg, and HBcAg have been widely used in clinical diagnosis and disease progression monitoring, a single effective serological marker for definitive infection diagnosis remains elusive. Conversely, advancements in genetics and proteomics technologies have revealed associations between DNA methylation and oxidative stress, immune metabolism, and signal transduction pathways, highlighting its significant role in liver diseases. Therefore, recognizing the epigenetic characteristics underlying disease development can serve as an important tool for disease diagnosis, understanding pathogenesis mechanisms, and predicting prognosis.

FGF21 is a stress-responsive hepatic factor primarily expressed in the liver, believed to play a protective role in

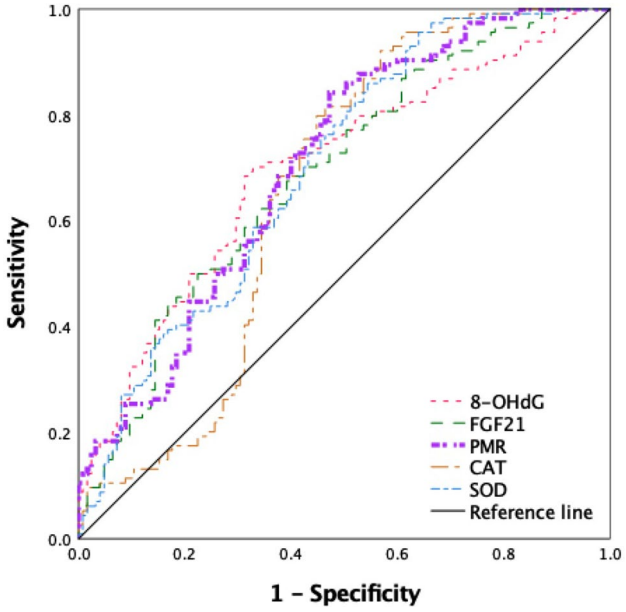

Fig. 5 The diagnostic value of FGF21 promoter methylation levels in HBeAg(+)CHB. The ROC of FGF21 promoter methylation level was better than 8-OHdG, CAT, SOD in the oxidative stress response in CHB patients

maintaining liver homeostasis when exposed to various stimuli. Studies [30–32] have demonstrated that FGF21 possesses the ability to directly and indirectly reduce the incidence of NAFLD through its antioxidant and anti-inflammatory effects, as well as its impact on lipid profile and adiponectin expression. It can also serve as a diagnostic biomarker for NAFLD. Furthermore, FGF21 regulates adipocyte energy homeostasis by activating AMPK and Sirtuin 1, leading to enhanced mitochondrial oxidation function [33]. Additionally, treatment with FGF21 has been shown to prevent fatty liver progression and reverse the advancement of NAFLD in mice [34]. Upregulation of FGF21 also aids in preventing fat deposition in the liver, thereby reducing inflammation and fibrosis within the organ [35]. Liver steatosis was reversed in mice using FGF21 analogs [36]. In mouse models of NASH, utilizing leptin-deficient mice and a methionine- and choline-deficient diet, an analogue of FGF21 reversed liver inflammation and fibrosis [31, 37]. Alcohol exposure can result in liver fat accumulation, endoplasmic reticulum stress, and inflammation which triggers the production of FGF21. Research indicates that alcohol-induced FGF21 expression is an adaptive response in the liver due to lipid dysregulation [38]. This process inhibits lipid synthesis while increasing serum levels of FGF21 [39]. As a significant indicator of liver injury, extensive documentation exists regarding FGF21's involvement in metabolic liver diseases such as alcoholic liver disease and non-alcoholic fatty liver disease; however, its role remains unexplored concerning HBV occurrence and development. Therefore, it is necessary to further investigate the value of FGF21 in HBV infection-associated liver disease.

In this study, we reported that the FGF21 mRNA level in CHB patients with HBeAg(+) was higher than that in CHB patients with HBeAg(-), which may be due to the compensatory increase, and we also detected the FGF21 promoter methylation level. We observed a notable decrease in methylation levels of the FGF21 promoter in CHB patients compared to HCs ($P<0.05$), with lower levels detected in HBeAg(+) CHB patients than in HBeAg(-) counterparts. We analyzed the correlation between the methylation level of FGF21 promoter and viral load and liver function, and the results showed that the methylation level of FGF21 promoter was significantly negatively correlated with the levels of HBeAg, HBeAb, ALT and AST. This suggests that this phenomenon may be involved in the immune activation process of liver injury and antiviral therapy in CHB patients, although there is no significant negative correlation with HBV-DNA and HBsAg. At the same time, we detected the oxidative stress levels of CHB and HCs. We found that the oxidative stress in CHB patients was more intense, and the oxidative stress level in HBeAg(+) patients was higher than that in HBeAg(-) CHB patients. This may be related to the imbalance in the regulation of free radicals and antioxidants in patients with chronic hepatitis B. In patients with CHB, immune dysfunction, enhanced inflammatory response, immune complex formation, complement activation and collagen fiber formation can stimulate the increase of ROS production, which can lead to the formation of lipid peroxide in liver cells. The longer ROS generation and clearance in the body were out of balance, the weaker the antioxidant defense and protection function of tissue cells and the more severe the liver injury. Next, we conducted correlation analysis on the methylation level of FGF21 promoter and oxidative stress indexes, and found that the methylation level of FGF21 promoter was negatively correlated with the oxidation index 8-OHdG ($P<0.05$), and positively correlated with the antioxidant indexes of CAT and SOD. We speculated that when the methylation level of FGF21 promoter is reduced, the expression of this gene is increased, resulting in the decline of its antioxidant function, the accumulation of ROS in vivo or in cells, and the decreased protective effect on the liver, which may aggravate oxidative damage. At the same time, due to the occurrence of viral replication and immune inflammation, the oxidative stress of the body is intense, and the compensatory increase of FGF21 mRNA expression is also caused. The possible mechanism by which oxidative stress affects the methylation of FGF21 is that oxidative stress leads to the destruction of protein structure, thus mediating the increase of transmethylase level and the enhancement of activity,

but the specific mechanism needs further study [40]. In addition, we compared the ability of different indicators to predict the occurrence of oxidative stress in the body. Interestingly, we found that FGF21 promoter methylation has a good evaluation effect on the prediction ability of oxidative stress, which may be an important marker to measure the balance between oxidation and antioxidant in the body. In summary, the occurrence of oxidative stress is closely related to the course and progression of HBV infection and antiviral therapy. As an important indicator of antiviral efficacy, the balance between oxidation and antioxidant in the body under different HBeAg serological states is crucial to the course and treatment of HBV infection. The detection of FGF21 promoter methylation level can help us to judge the oxidative stress level and antiviral efficacy of CHB patients.

There were also some limitations affecting our study. Firstly, it is a single-center cohort study with a relatively small number of CHB patients. Future studies will be conducted in larger, multi-center, prospective cohorts. Secondly, due to the outpatient nature of most patients and the unavailability of liver biopsy specimens, direct detection of FGF21 expression in liver tissue was not possible. Finally, we only preliminarily examined the relationship between FGF21 promoter methylation levels and oxidative stress in CHB patients with different HBeAg serologic statuses, and HBeAg seroconversion needs to be evaluated in the long term. In further studies, we will further explore the antioxidant mechanism of FGF21 in the body and dynamically observe the changes of HBeAg, in order to verify the potential value of FGF21 in antiviral therapy and the conversion of HBsAg and HBeAg.

Conclusion

In conclusion, this study found novel findings on the methylation status of the FGF21 promoter in CHB patients with varying HBeAg serological states. Our results indicate a correlation between FGF21 promoter methylation levels and oxidative stress in CHB patients. Additionally, we observed decreased FGF21 promoter methylation in HBeAg(+) CHB patients compared to HBeAg(-) patients, suggesting that HBV replication, imbalanced oxidative and antioxidant stress, and compensatory changes may contribute to this alteration as a protective mechanism for immune system regulation. Therefore, reduced methylation of the FGF21 promoter holds potential as an indicator for assessing oxidative stress severity and predicting HBeAg seroconversion during antiviral therapy.

Abbreviations

HBV	Hepatitis B virus
FGF21	Fibroblast growth factor 21
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
8- OHdG	9- 8-hydroxy-2'-deoxyguanosine
SOD	Superoxide dismutase
CAT	Catalase

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Author contributions

Kai Wang designed the study and carried it out. Xue Li, Pei Liu, Zhaohui Wang, Xuefei Wei, Shuai Gao, YuChen Fan and Huihui Liu collect data. Xue Li conducted the statistical analysis and drafted the manuscript. All the authors read and approved the manuscript.

Data availability

No datasets were generated or analysed during the current study.

Declarations

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).

Competing interests

The authors declare no competing interests.

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References

1. Khetsuriani N, Lesi O, Desai S, et al. Progress Toward the Elimination of Mother-to-Child Transmission of Hepatitis B Virus-Worldwide, 2016–2021. MMWR Morb Mortal Wkly Rep. 2022;71(30):958–63. https://doi.org/10.15585/mmwr.mm7130a2.
2. Salama II, Sami SM, Salama SI, et al. Current and novel modalities for management of chronic hepatitis B infection. World J Hepatol. 2023;15(5):585–608. https://doi.org/10.4254/wjh.v15.i5.585.
3. Lu F, Wang J, Chen X, et al. Potential use of serum HBV RNA in antiviral therapy for chronic hepatitis B in the era of nucleos(t)ide analogs. Front Med. 2017;11(4):502–8. https://doi.org/10.1007/s11684-017-0590-z.
4. Mansouri A, Gattolliat CH, Asselah T. Mitochondrial Dysfunction and Signaling in Chronic Liver Diseases. Gastroenterology. 2018;155(3):629–47. https://doi.org/10.1053/j.gastro.2018.06.083.
5. Yang F, Yan S, He Y, et al. Expression of hepatitis B virus proteins in transgenic mice alters lipid metabolism and induces oxidative stress in the liver. J Hepatol. 2008;48(1):12–9. https://doi.org/10.1016/j.jhep.2007.06.021.
6. Popa GL, Popa MI. Oxidative Stress in Chronic Hepatitis B-An Update. Microorganisms. 2022;10(7):1265. https://doi.org/10.3390/microorganisms10071265.
7. Zapotoczny B, Braet F, Kus E, et al. Actin-spectrin scaffold supports open fenestrae in liver sinusoidal endothelial cells. Traffic. 2019;20(12):932–42. https://doi.org/10.1111/tra.12700.
8. Pollicino T, Caminiti G. HBV-Integration Studies in the Clinic: Role in the Natural History of Infection. Viruses. 2021;13(3):368. https://doi.org/10.3390/v13030368.
9. Luangmonkong T, Suriguga S, Mutsaers HAM, et al. Targeting Oxidative Stress for the Treatment of Liver Fibrosis. Rev Physiol Biochem Pharmacol. 2018;175:71–102. https://doi.org/10.1007/112_2018_10.

10. Liu ZY, Luo Y, Fang AP, et al. High serum fibroblast growth factor 21 is associated with inferior hepatocellular carcinoma survival: A prospective cohort study. LIVER INT. 2021;42(3):663–73. https://doi.org/10.1111/liv.15100.
11. Chen MM, Hale C, Stanislaus S, et al. FGF21 acts as a negative regulator of bile acid synthesis. J ENDOCRINOL. 2018;237(2):139–52. https://doi.org/10.1530/JOE-17-0727.
12. Zhang J, Gupte J, Gong Y, et al. Chronic Over-expression of Fibroblast Growth Factor 21 Increases Bile Acid Biosynthesis by Opposing FGF15/19 Action. EBioMedicine. 2016;15:173–83. https://doi.org/10.1016/j.ebiom.2016.12.016.
13. Suomalainen A. Fibroblast growth factor 21: a novel biomarker for human muscle-manifesting mitochondrial disorders. Expert Opin Med Diagn. 2013;7(4):313–7. https://doi.org/10.1517/17530059.2013.812070153.
14. Liang P, Zhong L, Gong L, et al. Fibroblast growth factor 21 protects rat cardiomyocytes from endoplasmic reticulum stress by promoting the fibroblast growth factor receptor 1-extracellular signal–regulated kinase 1/2 signaling pathway. INT J MOL MED. 2017;40(5):1477–85. https://doi.org/10.3892/ijmm.2017.3140.
15. Luo Y, Ye S, Chen X, et al. Rush to the fire: FGF21 extinguishes metabolic stress, metaflammation and tissue damage. CYTOKINE GROWTH F R. 2017;38:59–65. https://doi.org/10.1016/j.cytogfr.2017.08.001.
16. Sarin SK, Kumar M, Lau GK, et al. Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. HEPATOL INT. 2015;10(1):1–98. https://doi.org/10.1007/s12072-015-9675-4.
17. Xiang L, Chen LM, Zhai YJ, et al. Hypermethylation of secreted frizzled related protein 2 gene promoter serves as a noninvasive biomarker for HBV-associated hepatocellular carcinoma. Life Sci. 2021;270:119061. https://doi.org/10.1016/j.lfs.2021.119061.
18. Li LC, Dahiya R. MethPrimer: designing primers for methylation PCRs. Bioinformatics. 2002;18(11):1427–31. https://doi.org/10.1093/bioinformatics/18.11.1427.
19. Gao S, Sun FK, Fan YC, et al. Aberrant GSTP1 promoter methylation predicts short-term prognosis in acute-on-chronic hepatitis B liver failure. Aliment Pharmacol Ther. 2015;42(3):319–29. https://doi.org/10.1111/apt.13271.
20. Sadasivam N, Kim YJ, Radhakrishnan K, et al. Oxidative Stress, Genomic Integrity, and Liver Diseases. Molecules. 2022;27(10):3159. https://doi.org/10.3390/molecules27103159.
21. Campbell EL, Colgan SP. Control and dysregulation of redox signalling in the gastrointestinal tract. Nat Rev Gastroenterol Hepatol. 2019;16(2):106–20. https://doi.org/10.1038/s41575-018-0079-5.
22. Seen S. Chronic liver disease and oxidative stress - a narrative review. Expert Rev Gastroenterol Hepatol. 2021;15(9):1021–35. https://doi.org/10.1080/17474124.2021.1949289.
23. Niu D, Zhang J, Ren Y, et al. HBx genotype D represses GSTP1 expression and increases the oxidative level and apoptosis in HepG2 cells. Mol Oncol. 2009;3(1):67–76. https://doi.org/10.1016/j.molonc.2008.10.002.
24. Loureiro D, Tout I, Narguet S, et al. Mitochondrial stress in advanced fibrosis and cirrhosis associated with chronic hepatitis B, chronic hepatitis C, or nonalcoholic steatohepatitis. Hepatology. 2023;77(4):1348–65. https://doi.org/10.1002/hep.32731.
25. Iloeje UH, Yang HI, Su J, Risk Evaluation of Viral Load Elevation and Associated Liver Disease/, Cancer- HBV, the REVEAL-HBV) Study Group. (. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. Gastroenterology. 2006;130(3):678 – 86. https://doi.org/10.1053/j.gastro.2005.11.016
26. Zhu M, Wang H, Lou T, et al. Current treatment of chronic hepatitis B: Clinical aspects and future directions. Front Microbiol. 2022;13:975584. https://doi.org/10.3389/fmicb.2022.975584.
27. Chien RN, Liaw YF. Current Trend in Antiviral Therapy for Chronic Hepatitis B. Viruses. 2022;14(2):434. https://doi.org/10.3390/v14020434.
28. Alexopoulou A, Karayiannis P. HBeAg negative variants and their role in the natural history of chronic hepatitis B virus infection. World J Gastroenterol. 2014;20(24):7644–52. https://doi.org/10.3748/wjg.v20.i24.7644.
29. Hu Y, Wu X, Ye Y, et al. Liver histological reversibility of lamivudine in treatment-naïve children with chronic hepatitis B: a retrospective cohort study from a single center Shanghai China. Transl Pediatr. 2023;12(6):1121–9. https://doi.org/10.21037/tp-22-496.
30. Tabari FS, Karimian A, Parsian H, et al. The roles of FGF21 in atherosclerosis pathogenesis. Rev Endocr Metab Disord. 2019;20(1):103–14. https://doi.org/10.1007/s11154-019-09488-x.
31. Keinicke H, Sun G, Mentzel CMJ, et al. FGF21 regulates hepatic metabolic pathways to improve steatosis and inflammation. Endocr Connect. 2020;9(8):755–68. https://doi.org/10.1530/EC-20-0152.
32. Rusli F, Deelen J, Andriyani E, et al. Fibroblast growth factor 21 reflects liver fat accumulation and dysregulation of signalling pathways in the liver of C57BL/6J mice. Sci Rep. 2016;6:30484. https://doi.org/10.1038/srep30484.
33. Chau MD, Gao J, Yang Q, et al. Fibroblast growth factor 21 regulates energy metabolism by activating the AMPK-SIRT1-PGC-1alpha pathway. P NATL ACAD SCI USA. 2010;107(28):12553–8. https://doi.org/10.1073/pnas.1006962107.
34. Ferrer-Curriu G, Guitart-Mampel M, Rupérez C, et al. The protective effect of fibroblast growth factor-21 in alcoholic cardiomyopathy: a role in protecting cardiac mitochondrial function. J Pathol. 2021;253(2):198–208. https://doi.org/10.1002/path.5573.
35. Yan H, Xia M, Chang X, et al. Circulating fibroblast growth factor 21 levels are closely associated with hepatic fat content: a cross-sectional study. PLoS ONE. 2011;6(9):e24895. https://doi.org/10.1371/journal.pone.0024895.
36. Tucker B, McClelland RL, Allison MA, et al. Relationship of fibroblast growth factor 21 levels with inflammation, lipoproteins and non-alcoholic fatty liver disease. Atherosclerosis. 2020;299:38–44. https://doi.org/10.1016/j.atherosclerosis.2020.03.009.
37. Stefan N, Schick F, Birkenfeld AL, et al. The role of hepatokines in NAFLD. Cell Metab. 2023;35(2):236–52. https://doi.org/10.1016/j.cmet.2023.01.006.
38. Liu Y, Zhao C, Xiao J, et al. Fibroblast growth factor 21 deficiency exacerbates chronic alcohol-induced hepatic steatosis and injury. Sci Rep. 2016;6:31026. https://doi.org/10.1038/srep31026.
39. Raptis DD, Mantzoros CS, Polyzos SA. Fibroblast Growth Factor-21 as a Potential Therapeutic Target of Nonalcoholic Fatty Liver Disease. Ther Clin Risk Manag. 2023;19:77–96. https://doi.org/10.2147/TCRM.S352008.
40. Prasad R, Singh T, Katiyar SK. Honokiol inhibits ultraviolet radiation-induced immunosuppression through inhibition of ultraviolet-induced inflammation and DNA hypermethylation in mouse skin. Sci Rep. 2017;7(1):1657. https://doi.org/10.1038/s41598-017-01774-5.

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