Japanese Journal of Gastroenterology and Hepatology

Research Article ISSN: 2435-1210 | Volume 8

Association of IL-32 Isoforms Responses with Pegifn Therapy in Chronic HBV Infection

Yongping Liu[1,3], Ying Meng[1], Jin Xu[1], Yinghui Li[1], Xiaoying Wu[1], Yaqun Li[2], Yaojie Shen[3*] and Yan Liu[1]

[1]Department of Hepatology, Affiliated Hangzhou Xixi Hospital, Zhejiang University School of Medicine, Hangzhou, China

[2]Department of Infectious Diseases, Huashan Hospital, Shanghai Medical College, Fudan University, Shanghai, China

[3]Department of Pharmacy, Huashan Hospital, Shanghai Medical College, Fudan University, Shanghai, China

***Corresponding author:**

Yaojie Shen,
Department of Pharmacy, Huashan Hospital, Shanghai Medical College, Fudan University, Shanghai, China, E-mail: shenyaojie@fudan.edu.cn

Received: 10 Apr 2022
Accepted: 09 May 2022
Published: 13 May 2022
J Short Name: JJGH

Citation:

Yaojie Shen. Association of IL-32 Isoforms Responses with Pegifn Therapy in Chronic HBV Infection. J Gstro Hepato. V8(17): 1-8

Keywords:

IL-32 isoform; Chronic hepatitis B; HBV-related liver diseases; Virologic response

1. Abstract

Interleukin-32 is a proinflammatory cytokine with immune protective effect. IL-32γ mediates antiviral activity of cytokines against HBV. However, the dynamic expression level of IL-32 isoforms in CHB and whether they are affected by drug therapy is not clear. Here we show that the persistent increased expression of plasma IL-32 is related to the progression of HBV-related liver diseases (from CHB, HBV-LC, HCC to HBV-ACLF). And by detecting the differential expression of PBMCs IL-32 mRNA and its transcripts variants, IL-32 was decreased in CHB patients with PegIFN therapy than in the health donor and the initial CHB patients without treatment. But for its transcript variants, IL-32α and IL-32ε were overexpressed in CHB patients and low expression in CHB with interferon treatment. Compared with healthy controls, IL-32β and IL-32δ were significantly expressed in the CHB patients with interferon therapy and followed by in the initial CHB, while IL-32ζ and IL-32γ were no signiant expression unless IL-32ζ was overexpressed in treated CHB patients. Collectively, IL-32 isoforms plays an important role in patients with CHB and interferon therapy. It also can be used as a marker for the diagnosis of interferon therapy and prediction of sustained virologic response in patients with chronic hepatitis B.

2. Introduction

Hepatitis B virus (HBV) infection is a major epidemic disease that affects people's health all over the world. WHO reported that there are about 250 million people with chronic HBV infection worldwide, and more than 0.9 million people die from HBV infection-related diseases every year. Persistent HBV infection may lead to different outcomes of the disease, from HBV carrier to chronic active hepatitis, liver fibrosis, and even liver cirrhosis (HBV-LC) and hepatocellular carcinoma, depending on host and viral factors [1]. However, so far, the pathogenesis of chronic HBV infection has not been fully elucidated. The clearance of HBV is closely related to the immune response to virus-encoded proteins [2]. Nucleoside analogues can inhibit the formation of viral particles in chronic hepatitis B, but cannot eliminate the stable presence of cccDNA in hepatocytes. Pegylated interferon plays an antiviral effect mainly through immune regulation, which can effectively reduce the incidence of liver cirrhosis and liver cancer. However, it has serious side effects and it is difficult to achieve a functional cure by single drug treatment [3-5]. It has been found that a variety of cytokines such as TNF-α and IFN-γ are involved in the early host defense against pathogen infection and the regulation of hepatocyte inflammation in patients with HBV infection, and play an important role in the elimination of acellular pathological changes caused by HBV infection [6-8]. IL-32 has been found as a pro-inflammatory cytokine secreted by NK cells and activated by MAPK and NF-κB signaling pathways [9]. IL-32 exists in numerous splice variants, mainly contains 6 different isoforms of IL-32 are characterized: IL-32α, IL-32β, IL-32γ, IL-32δ, IL-32ε and IL-32ζ [10]. IL-32 is involved in the response to different viruses, such as influenza virus, HIV, EBV and HBV. IL-32γ may participate in the specific mechanism of acellular cytopathic virus clearance. It is considered that IL-32γ, the most bioactive secretory subtype of IL-32, inhibits the transcription and replication of HBV by down-regulating the activity of liver transcription factors in hepatocytes [11]. However, the expression of IL-32 and its various isoforms in HBV-related liver diseases has not been systematically described. It has been reported that IL-32 in plasma is not consistent with its mRNA expres-

sion at the transcriptional level of PBMCs [12]. Also, the increased IL-32 was associated with HCC and related to liver inflammation in HBV-ACLF [13]. But, it is not clear which specific isoform plays a role function or different isoforms perform different functions. In this study, we examined the plasma IL-32 expression level in HBV-related liver diseases and their relationships. Furthermore, we detected the IL-32 and its transcript variants mRNA level in PBMCs of CHB patients with or without pegylated interferon-α2a treatment. We analyzed the association of IL-32 isoforms with virologic response to interferon therapy and explored the potential mechanism in Chronic hepatitis B patients.

3. Materials and Methods

3.1. Subjects

A total of 140 patients with hepatitis B diseases, including CHB, HBV-LC, HCC and HBV-ACLF. At the same time, 80 volunteers were included as healthy controls. All the patients were enrolled from Hangzhou Xixi hospital, Zhejiang, China between January 2018 to December 2020. All the cases were diagnosed in accordance with the criteria of guidelines which were detailed previously [14,15]. Other hepatitis virus infections, complicated with autoimmune hepatitis and alcoholic liver disease, were excluded. All peripheral blood samples of the enrolled patients and healthy donors were collected with written, informed consent and in accordance with the guidelines of the institutional review board.

3.2. Laboratory Assay

The results of liver function (ALT, AST and Albumin), Serum HBV DNA load and other HBV serological markers (HBsAg and HBeAg) were collected from blood routine examination reports of patients.

3.3 Enzyme-Linked Immunosorbent Assay (ELISA)

The expression level of IL-32 in plasma was detected by ELISA. 6 ml heparin anticoagulant peripheral blood of patients and healthy volunteers were collected and centrifuged at RT 3000rpm for 10min. Separated plasma was transferred to cryovial tube with pap straw and stored at -80°C. The plasma IL-32 level was detected by ELISA kit (ab267616, Abcam). Samples were taken out in advance and thawed at room temperature. After 1000rpm centrifugation for 5min, 100μl/tube plasma and all reagents were prepared according to the kit instructions. The standard sample was diluted, and the experiment was carried out strictly according to the steps. Finally, the plate was used to detect at OD450nm by SynergyTM2 (BioTek, USA). The standard curve was drawn and the IL-32 level of plasma samples was calculated.

3.4. Isolation of Pbmcs by Density Gradient Centrifugation

Plasma samples of patients were collected and assayed for IL-32 ELISA or stored at -80°C. Two tubes of 4 ml RPMI 1640 medium and one tube of 4ml Lympholyte®-H cell separation media (Cederlane) were prepared. Cell pellets of the middle lymphocyte layer were absorbed and transferred into a 4 ml RPMI 1640 medium tube with pap straw, slowly spread (2:1) into 4ml lymphocyte separation media tube and density gradient centrifugation for 20min. Lymphocyte cells were collected and transferred into another 4ml RPMI 1640 tube. After centrifugation and washing twice, PBMCs were harvested in 1mlTRIzol Reagent (Invitrogen, Thermo Fisher) and transferred into a 2ml cryopreservation tube for cryopreservation.

3.5. RNA Extraction, Reverse Transcription and Quantitative Real-Time PCR (Qrt-PCR)

Extraction of total RNA using TRIzol and Phenol-chloroform according to the manufacturer's instructions. After washing 75% alcohol twice and drying RNA, the total RNA was dissolved with the RNase-free water. The RT-PCR was carried out by adding samples to the reverse transcription reaction system recommended by PrimeScriptTM RT reagent Kit (RR047A, TAKARA) according to the instructions. The synthesized cDNA was amplified by PCR (ABI verity, Thermo Fisher) under the following conditions: incubation at 37°C for 60min and 95°C for 5min to inactivate reverse transcriptase. All cDNA preparations were stored at -20°C before further use. Real-time primers designed according to the splicing patterns of IL-32 mRNA and the sequences of six transcript variants reported in the NCBI database (Figure 1), which are IL-32, IL-32α, IL-32β, IL-32γ, IL-32δ, IL-32ε and IL-32ζ. Primers (Table1) and PCR reaction solutions were prepared according to the recommended reaction system and carried out on a 96-well plate. Real-time quantitative PCR amplification of gene relative expression was performed in an ABI PRISM 7500 sequence detection system (Life Technologies, Thermo Fisher) using a SYBR ®Premix ExTaq kit (Tli RNase H Plus) (RR420A, TAKARA). The reaction procedure followed as pre-denaturation at 95°C for 30 s and denaturation at 95°C for 5s. Annealing at 60°C for 34 s plus extension for 40 cycles. The results were expressed as an n-fold difference relative to the calibrator ($RQ = 2^{-\Delta\Delta Ct}$).

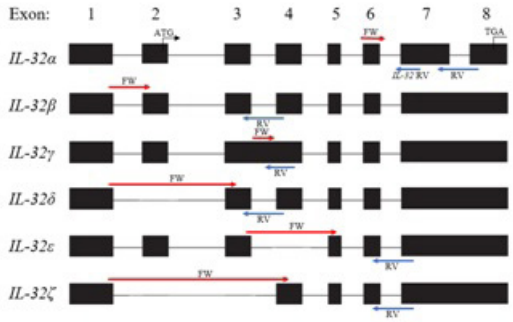

Figure 1: Schematic representation of the design of primers for the IL32 and its six transcript variants (α, β, γ, δ, ε and ζ). The red arrows indicate the forward primer and the blue arrows indicate the reverse primer.

Table 1: Sequences and fragment size of primers used in quantitative PCR experiments.

Target gene	Primer sequences (5'-3')	Size (bp)
IL-32	FW:5'-TGGCGGCTTATTATGAGGAGC-3'	78
	RV:5'-CTCGGCACCGTAATCCATCTC-3'	
IL-32α	FW:5'-TGGCGGCTTATTATGAGGAGC-3'	157
	RV:5'-CCGTAGGACTTGTCACAAAA-3'	
IL-32β	FW:5'-TCTGTCTCTCTCGGGCCTTGG-3'	109
	RV:5'-CTATGGCCTGGTGCATTCGG-3'	
IL-32γ	FW:5'-GTAATGCTCCTCCCTACTTCT-3'	160
	RV:5'-AAAATCTTTCTATGGCCTGGT-3'	
IL-32δ	FW:5'-TGTCTCTCTCGGGTCCTCTCTGAT-3'	64
	RV:5'-CTATGGCCTGGTGCATTCGG-3'	
IL-32ε	FW:5'-GAAGGCCCGAATGGTGATGTC-3'	104
	RV:5'-ACCTCTGTCTCTCTCGGCACCA-3'	
IL-32ζ	FW:5'-ACCTCTGTCTCTCTCGGCACCA-3'	167
	RV:5'-CTCTGGGTGCTGCTCCTCATAAT-3'	

3.6. Statistical Analysis

The data in accordance with normal distribution is expressed by mean ± standard deviation. SPSS Statistics 20 was used to analyze by two unpaired t tests. The comparison between the two groups is carried out by the Mann-Whitney test. The risk factors are analyzed by binary Logistic regression, and the ROC curve is drawn by GraphPad software. The area under the curve (AUC) was compared to the normal Z test. The differences were considered statistically significant at a value of $P<0.05$.

4. Results

4.1. Patient Characteristics

A total of 140 peripheral blood samples of patients with hepatitis B diseases (80 CHB patients, 20 HBV-LC, 20 HCC and 20 HBV-ACLF). Table 2 sets out the characteristics of the participating patients with HBV-related liver diseases about the liver function (ALT, AST and Albumin), serum HBV DNA load and other HBV serological markers (HBsAg and HBeAg). 80 healthy donors were also included as HC group.

Among 80 CHB patients, a total of 60 patients were selected from CHB group. 30 initial diagnostic chronic HBV infection who never received any antiviral therapy (CHB-N group, 22 HBeAg positive and 8 HBeAg negative), including 17 males and 13 females, aged from 29 to 53 years old, with an average age of 35.5 ±8.3 years. Another 30 patients with CHB were treated with long-acting interferon PegIFN-α2a (CHB-T group, 18 HBeAg positive and 12 HBeAg), including 19 males and 11 females, aged from 25 to 48 years old, with an average age of 33.2 ±6.3years. At the same time, 30 healthy volunteers were selected as controls.

In CHB-T group, peripheral blood collection time is the end point of PegIFN-α2a treatment which refers to the achievement of virologic response at 48 weeks (HBeAg disappearance and HBV DNA not detected or under the cutoff), including 10 patients with sustained virologic responders (SVR group) and 20 patients with non-responders (NR group).

Table 2: Patient characteristics of HBV-related liver diseases.

Characteristics	CHB (n=80)	HBV-LC (n=20)	HBV-HCC (n=20)	HBV-ACLF (n=20)	HC (n=80)
Age(years)	37.7 ± 10.3	53.1 ± 3.0	49.5 ± 3.4	61.4 ± 10.7	32.4 ± 7.8
Gender (M/F)	55/25	13/7	18/2	16/4	46/34
ALT (U/L)	238.29 ± 320.82	87.30 ± 25.71	522.50 ± 120.30	246.34 ± 29.34	38.17 ± 13.48
AST (U/L)	142.71 ±181.82	72.00 ± 18.16	521.50 ± 141.60	128.37± 31.29	33.53 ± 14.23
ALB (g/L)	42.43 ± 5.78	36.36 ± 3.79	34.40 ± 2.09	31.01 ± 1.37	36.86 ± 4.08
HBsAg (log IU/mL)	3.29 ± 1.31	2.84 ± 0.42	2.97 ± 0.56	2.79 ± 0.31	NE
HBeAg (S/CO)	457.41 ± 614.26	205.50 ± 138.90	1.49 ± 1.07	65.29 ± 36.60	NE
HBV DNA (log IU/mL)	5.79 ± 1.83	5.49 ± 0.41	5.34 ± 0.48	5.11 ± 0.35	NE

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALB, albumin; CHB, Chronic hepatis B; NE, not examined; HC, Healthy control.

4.2. Plasma IL-32 Expression Level Was Increased in HBV-Related Liver Dieases

IL-32 level of plasma detected by ELISA in CHB, HBV-LC, HCC and HBV-ACLF patients and healthy controls. The results of each group were 259.26 ±174.16 pg/ml, 385.84 ±142.36 pg/ml, 529.46 ±84.36 pg/ml and 883.17 ±189.34 pg/ml, respectively. They were significantly higher than those in healthy controls (89. 2±22.45) pg/ml (F=147.4, P<0.0001), and also there was a difference between each two groups (P<0.05). The amount of IL-32 level is shown in **Figure 2**.

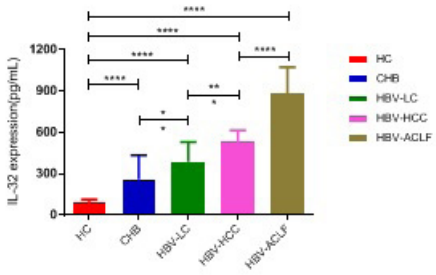

Figure 2: The comparison of plasma IL-32 levels in patients with CHB, HBV-LC, HBV-HCC, HBV-ACLF and healthy controls.

4.3. Pbmcs IL-32 And Its Isoforms Mrna Expression Level Was Associated with Pegifn Treatment in CHB Patients

PBMCs were isolated from all peripheral blood samples, and RNA was extracted and reverse transcribed into cDNA, IL-32 and its various transcripts mRNA level were determined by SYBR assay using real-time PCR. The majority of CHB patients showed that there was no significant in IL-32 mRNA expression between the CHB-N group and healthy controls, while the expression of IL-32 mRNA in the CHB-T group was significantly lower than that in healthy controls and CHB-N group (Figure 3A). It was considered statistically significant at a value of P<0.0001. To further investigate the role of decreased level of IL-32 mRNA in CHB patients with pegylated interferon therapy, IL-32 isoforms mRNA levels were determined. In an attempt to determine the most active isoform form of IL-32 in peripheral blood of CHB-N, CHB-T and HC groups, the gene expression levels of all IL-32 isoforms were compared (Figure 3B). In HC and CHB-N, IL-32γ and IL-32ε are the main active isoform. In the CHB-T group, the high expression of IL-32γ is the most active subtype, while the expression of IL-32ε is relatively low. However, results reveal that tested IL-32 isoforms, IL-32α, IL-32β, IL-32γ, IL-32δ, IL-32ε and IL-32ζ mRNA were expressed in the PBMCs of Chronic hepatitis B patients as well as in those of healthy donors. Compared with healthy controls, IL-32α and IL-32ε in patients with chronic hepatitis B were significantly higher, IL-32β and IL-32δ were significantly lower, IL-32γ and IL-32ζ were not significantly different. compared of the initial CHB patients without any treatment and the CHB patients with PegIFN treatment, IL-32α, IL-32β, IL-32δ, IL-32ε all decreased expression significantly, but IL-32ζ increased significantly, as long as IL-32γ had no significant difference (Figure 4A-F). These data pointed out that all isoforms of were significantly differential expressed in the PBMCs of CHB-T group and CHB-N group patients as compared with PBMCs from healthy donor controls. It may suggest that IL-32 isoforms of CHB patients involved in the defense against HBV infection in different ways and related to interferon therapy pathways. As mentioned above, the differential expression of IL-32 and its various transcripts was significantly in each group, suggesting the possibility of IL-32 isoforms could discriminate against the CHB treated or not. The efficacy of IL-32γ, IL-32δ and IL-32ζ was compared by calculating the AUC of these predictors. For predicting significant initial CHB, the AUC was 0.8580 of IL-32γ, which was shown to be significantly higher than that of IL-32δ (0.8149), and IL-32ζ (0.8185) all P < 0.05 (Figure 3G).

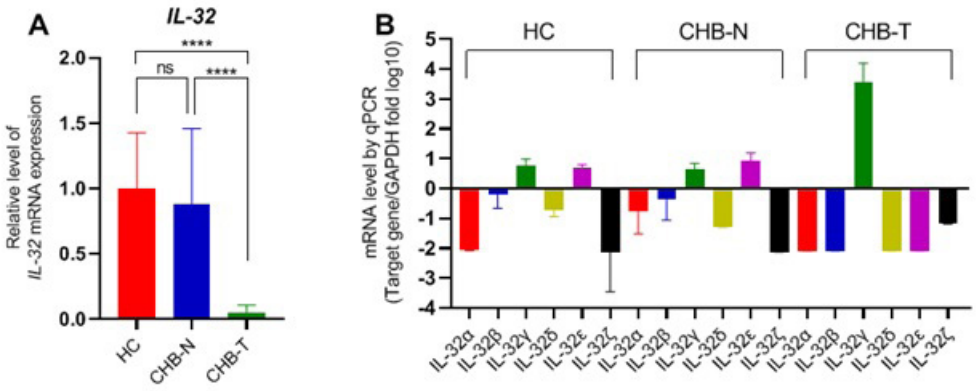

Figure 3: A, IL-32 mRNA expression after pegylated interferon therapy in CHB patients. B, Gene expression of IL-32 isoforms in PBMCs from CHB patients with or without treatment and healthy controls. The mRNAs of IL-32 isoforms were determined by real-time qRT-PCR using GAPDH as an endogenous control in comparative -ΔΔCT method.

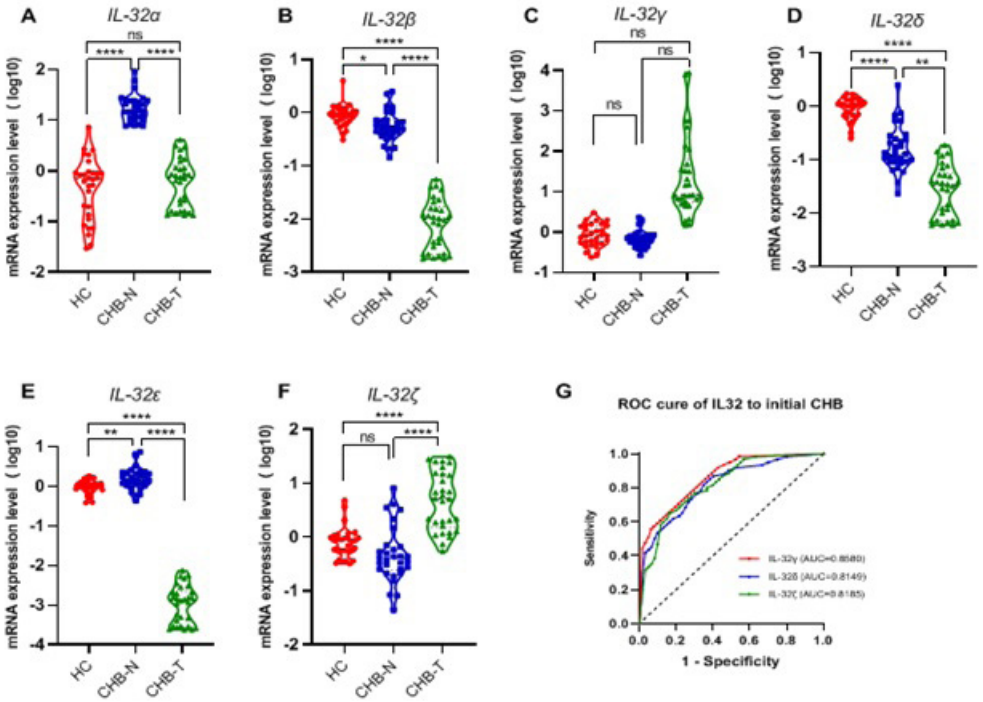

Figure 4: A-F, The expression of IL-32 transcript variants in CHB. qPCR analysis of IL-32α, IL-32β, IL-32γ, IL-32δ, IL-32ε and IL-32ζ mRNA levels in PBMCs from healthy donors and the group of patients with the diagnosis of CHB. Expression was assessed by qPCR (mean fold change ± SEM, n = 30. *P* value by unpaired t test). G, The ROC cure of IL-32γ, IL-32δ and IL-32ζ to CHB with PegIFN-α2a treatment.

4.4. IL-32γ Is A Predictor of Sustained Virologic Responses in Chronic HBC Infection During Interferon Treatment

According to the efficacy of interferon in the treatment of Chronic hepatitis B, 30 CHB patients treated with interferon were divided into sustained virology responses (SVR group, n=10) and non-responses (NR group, n=20). There was no significant difference in IL-32 and its forms between the two groups except IL-32γ. The expression level of IL-32γ in CHB-SVR group was significantly higher than that in CHB-NR group (P=0.0399) and extremely significantly higher than that in CHB-N group (P=0.0083). It suggested that the increased IL-32γmRNA expression level could distinguish the CHB-SVR from the CHB patients with PegIFN treatment (Figure 5A). However, for the ROC cure analysis of IL-32 isoforms, it was found that IL-32 and IL-32γ had a certain value of predicting SVR CHB, and the AUC was 0.5973 and 0.5734, respectively (Figure 5B).

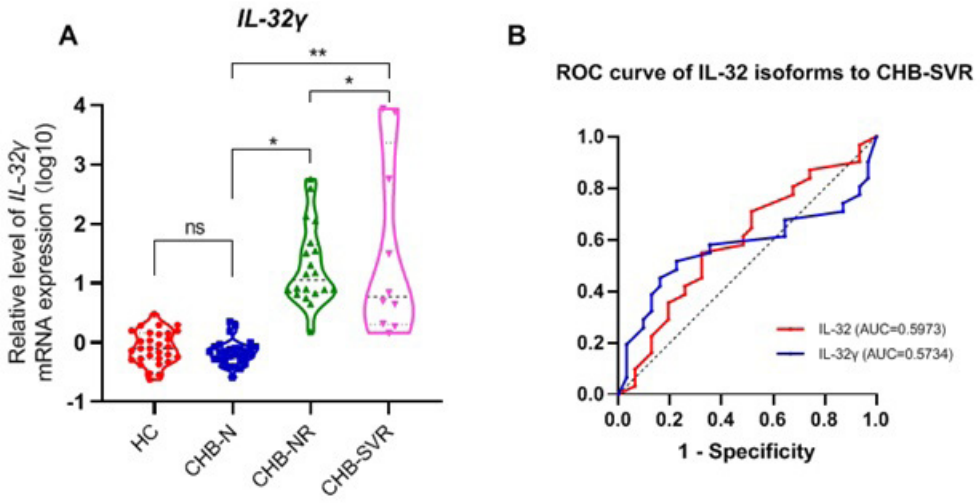

Figure 5: A The differential expression of IL32γ level in responses to the SVR patients with pegylated interferon therapy of CHB infection. B, The ROC cure of IL-32 isoforms to CHB-SVR.

5. Discussion

The condition and outcome of HBV infection are affected by many factors, which the host immune and cytokines play a role in regulating the immune response. It has been found that the HBV and its coding proteins can increase the expression of IL-32 in hepatocytes and the level of IL-32 in peripheral blood of chronic hepatitis B [18,19]. It is confirmed that HBV infection leads to the changes of IL-32 expression level in peripheral blood. In this study, the expression level of IL-32 in plasma gradually increased and there was a significant difference from healthy controls to chronic hepatitis B, liver cirrhosis, liver cancer and other hepatitis B virus-related liver diseases, with the development of the disease. It suggested that IL-32 may be involved in liver tissue injury and disease development in patients with HBV infection. IL-32, once named natural killer cell transcript 4 (NK4), has recently been recognized as an important pro-inflammatory cytokine [9]. It is mainly derived from NK cells, T cells, epithelial cells and monocytes, and plays an important role in innate immunity. IL-32 plays an immune protective role and may play a magnifying effect in inflammatory response [19]. IL-32 is related to the occurrence of a variety of diseases, and there is a variety of splicing isomerism in gene expression. At present, at least six different splicing forms have been found. IL-32 α, IL-32 β and IL-32 δ are all intracellular proteins. Many studies have shown that IL-32 γ has higher activity in inducing cytokine expression and antivirus activity than other isoforms [20]. IL-32 is mainly expressed by immune cells and also expressed in inflammatory tissues of some lesions. It can induce the expression of cytokines such as IL-1β, IL-6, IL-8, TNF-α, macrophage inflammatory protein 2 and so on [9].

Immunohistochemical detection showed that the expression of IL-32 in synovial fluid of rheumatoid arthritis was significantly higher than that of common arthritis, suggesting that it plays a special role in the pathology of rheumatoid arthritis. Further studies have revealed that IL-32 is closely related to the expression of inflammatory factors such as TNF-α, IL-1β and IL-18 in rheumatoid arthritis, so it is considered to be an important factor leading to autoimmune diseases [21]. Some studies have also shown that IL-32α is involved in the pathological process of inflammation because it induces the expression of a large number of inflammatory factors in intestinal inflammatory diseases. In studies of virus infection, the researchers found that the expression level of IL-32 in the serum of AIDS patients and influenza infected patients was significantly higher than that of normal people, which also suggests that it plays an important role in HIV and influenza virus infection and the disease process caused by HIV/AIDS. In hepatitis C studies, it is found that the expression of IL-32 can be induced by hepatitis C virus infection, and the expression level is closely related to hepatitis C virus-related hepatitis, liver cancer and liver fibrosis [22]. In this study, the expression level of IL-32 mRNA was detected in the PBMC of the collected clinical cohort samples. It was found that IL-32 mRNA was the same expression level in the initial CHB patients and the healthy donors. There was no significantly high expression of IL-32 in plasma, but the expression of IL-32 in treated patients was significantly low, which might be related to interferon therapy. Also, it might be related to the different expression of IL-32 isoforms, which of the different transcripts produced by the variable splicing of special cytokine IL-32. However, some studies have found that HBV can induce the high expression of IL-32 in Hepatocellular carcinoma cell lines. We suggested that IL-32 played an important role in the progression of hepatitis B infection diseases. But, it was not clear how IL-32 played a defensive role against the immune response, although HBV induced the high expression of IL-32 in plasma. Therefore, we speculated that it affected the progression of HBV-related liver diseases through the action of IL-32 and its various transcripts on hepatitis B virus. Previous studies focused on IL-32γ, which is the most active isoform with the highest biological activity and antiviral activity. Our study also confirmed the mechanism of IL-32γ in CHB. We found that

HBV infection induced over expression of IL-32α and IL-32ε, low expression of IL-32β and IL-32δ in patients with chronic hepatitis B. It suggested that IL-32 isoforms may be involved in the defense mechanism against HBV infection in the different pathways. However, in the chronic hepatitis B treated with interferon, the expression level of most IL-32 isoforms decreased except IL-32ζ was highly expressed. These results confirmed that the function of IL-32 in host defense may be responsible, and it was observed that different effects may involve various antivirus mechanisms. Many inflammatory cytokines are involved in virus clearance and immune response to HBV infection. Inflammatory cytokines play a key role in virus clearance of acute viral hepatitis. However, it is a pathogenic factor for chronic hepatitis because persistent chronic hepatitis can lead to liver fibrosis and cirrhosis [23]. In view of the possible pathogenesis of chronic hepatitis B, antiviral therapy plays an important role in controlling the progression of chronic hepatitis B [11]. There are mainly two kinds of antiviral drugs: nucleoside acid analogues (NA) and interferon (IFN). Pegylated interferon (PegIFN-α2a) is recommended for chronic hepatitis B. The seroconversion of the HBsAg is an ideal endpoint for the treatment of HBV infection [24]. To eliminate HBV, the body needs to produce an effective immune response. However, it is still unclear about the immune mechanism of the body in clearing the HBV. Kim et al [11] reported that IL-32γ functions as an intracellular effector in hepatocytes for suppressing HBV replication to implicate a possible mechanism of non-cytopathic viral clearance.

In this study, we found that IL-32γ mRNA expression level was a significant difference between CHB-SVR and CHB-NR compared with CHB-N ($P<0.05$). It was suggested that IL-32γ is associated with sustained virologic response to interferon therapy and has a certain predictive value. Additionally, somewhat unexpectedly, our study showed the IL-32 level detected in the clinical samples after PegIFN-α2a monotherapy were significantly decreased. However, only the IL-32γ was found to be extremely highly expressed through the detection of IL-32 isoforms. In addition to our belief that IL-32 detected by plasma ELISA is only a secreted protein expressed by IL-32 mRNA, and it has different functions of IL-32 isoforms. So, it is indicated that IL-32γ plays a positive immune regulation, and may be involved in the body's immune response to clear the HBsAg and/or suppress HBV replication. The study of Li et al. [25] believed that HBV enhanced its binding to the IL-32 promoter by activating the NF-kB signaling pathway, thereby inducing the transcription level of IL-32. The NF-ϰB signaling pathway inhibitor Bay-11-7082 could effectively prevent the HBV-induced IL-32 expression was blocked, demonstrating that the NF-ϰB pathway plays an important role in mediating IL32 expression during HBV infection. When cells and organisms are infected by HBV, the receptor cells can secrete interferon to protect other cells against the invasion of various viruses. The antiviral mechanism of PegIFN is through binding to the specific interferon type I receptor (IFNAR) on the cell membrane, mainly through the Jak-STAT signaling pathway, triggering cascade signal amplification, and transmitting the signal into the nucleus [26]. Interferon also can enhance the activity of NF-ϰB by inducing MyD88, an important component of the Toll-like receptor (TLRs) signaling cascade amplification system, thereby inhibiting HBV transcription and replication [27]. Therefore, whether interferon may have any targeted regulation on the transcriptional splicing of IL-32 through the NF-kB signaling pathway. So that IL-32γ isoform with antiviral effect can be screened by the long-term treatment of interferon. However, it should be more experimental evidence to further confirm. In sum, IL-32 might be involved in the process of liver tissue injury and disease development in patients with chronic hepatitis B. It is an important cytokine in the pathogenesis of HBV-related diseases. IL-32γ and other isoforms have potential clinical values in the diagnosis of HBV infection. At the same time, IL-32γ may play a protective role in the sustained virologic response of CHB patients who are treated with interferon.

6. Conclusion

In present study, we analyzed plasma IL-32 levels in patients with HBV-related diseases and mRNA levels of IL-32 isoforms in PBMCs of patients with/without PegIFN therapy. We found that on of IL32γ level in responses to the SVR patients with pegylated interferon therapy of CHB. We concluded that IL-32γ plays an important role in CHB patients and that IL-32 isoforms can be used as a marker of sustained virologic response in PegIFN-treated patients.

7. Acknowledgment

This work was supported by the Hangzhou Health Science and Technology General Plan (OO20190318 and A20220228).

References

1. EASL. Clinical Practice Guidelines on the management of hepatitis B virus infection. J Hepatology. 2017; 67: 370-398.
2. Maini MK, Boni C, Lee CK. The role of virus-specific CD8(+) cells in liver damage and viral control during persistent hepatitis B virus infection. J Exp Med. 2000; 191: 1269-1280.
3. Trepo C, Chan HL, Lok A. Hepatitis B virus infection. Lancet. 2014; 384: 2053-2063.
4. Lok AS, Zoulim F, Dusheiko G. Hepatitis B cure: From discovery to regulatory approval. Hepatology. 2017; 67: 847-861.
5. Zoulim F, Lebosse F, Levrero M. Current treatments for chronic hepatitis B virus infections. Curr Opin Virol. 2016; 18: 109-116.
6. Janovec V, Hodek J, Clarva K. Toll-like receptor dual-acting agonists are potent inducers of PBMC-produced cytokines that inhibit hepatitis B virus production in primary human hepatocytes. Sci Rep. 2020; 10: 12767.
7. Pais R, Charlotte F, Fedchuk L. A systematic review of follow-up biopsies reveals disease progression in patients with non-alcoholic fatty liver. J Hepatol. 2013; 59: 550-556.
8. Pasquetto V, Wieland SF, Uprichard SL. Cytokine-sensitive replication of hepatitis B virus in immortalized mouse hepatocyte cultures. J Virol.

2002; 76: 5646-5653.

9. Kim SH, Han SY, Azam T. Dinarello CA. Interleukin-32: a cytokine and inducer of TNF-alpha. Immunity. 2005; 22: 131-142.
10. Kang JW, Park YS, Lee DH. Interaction network mapping among IL-32 isoforms. Biochimie. 2014; 101: 248-251.
11. Kim DH, Park ES, Lee AR. Intracellular interleukin-32γ mediates antiviral activity of cytokines against hepatitis B virus. Nat Commun. 2018; 9: 3284.
12. Netea MG, Azam T, Lewis EC. Mycobacterium tuberculosis induces interleukin-32 production through a caspase-1/IL-18/interferon--dependent mechanism. PLoS Med. 2006; 3: e277.
13. Zhou Y, Bao J, Pan X. NKP30-B7-H6 Interaction Aggravates Hepatocyte Damage through Up-Regulation of Interleukin-32 Expression in Hepatitis B Virus-Related Acute-On-Chronic Liver Failure. PLOS One. 2015; 10: e0134568.
14. Martin P, Nguyen MH, Dieterich DT. Treatment Algorithm for Managing Chronic Hepatitis B Virus Infection in the United States: 2021 Update. Clin Gastroenterol and Hepatol. 2021; S1542-3565(21)00818-1.
15. Ma N, Xu M, Dong Y. Genetic variants in IL-33 and IL1RL1 genes confer susceptibility to HBV-related liver cirrhosis in Chinese Han population. Infect Genet Evol. 2021; 94: 104983.
16. Liu CJ, Chen DS, Chen PJ. Epidemiology of HBV infection in Asian blood donors: emphasis on occult HBV infection and the role of NAT. J Clin Virol. 2006; 1: S33-44.
17. Harvala H, Reynolds C, Gibney Z. Hepatitis B infections among blood donors in England between 2009 and 2018: Is an occult hepatitis B infection a risk for blood safety? Transfusion. 2021; 61: 2402-2413.
18. Xu Q, Pan X, Shu X. Increased interleukin-32 expression in chronic hepatitis B virus-infected liver. J Infect, 2012; 65: 336-342.
19. Tian Z, Shen Y, Li X. Increased interleukin-32, interleukin-1, and interferon-γ levels in serum from hepatitis B patients and in HBV-stimulated peripheral blood mononuclear cells from healthy volunteers. J Infect Public Health. 2019; 12: 7-12.
20. Li W, Sun W, Liu L. IL-32□ A Host Proinflammatory Factor against Influenza Viral Replication Is Upregulated by Aberrant Epigenetic Modifications during Influenza A Virus Infection. The Journal of Immunology. 2010; 185: 5056-5065.
21. Joosten LAB. IL-32, a proinflammatory cytokine in rheumatoid arthritis. Proceedings of the National Academy of Sciences. 2006; 103: 3298-3303.
22. Moschen AR, Fritz T, Clouston AD. Interleukin-32: a new proinflammatory cytokine involved in hepatitis C virus-related liver inflammation and fibrosis. Hepatology. 2011; 53: 1819-1829.
23. Li T, Yang Y, Zhou G. Immune suppression in chronic hepatitis B infection associated liver disease: A review. World J Gastroenterol. 2019; 21: 3527-3537.
24. Visentini M, Casato M. HBV messing with the B-cell genome leads to DLBCL. Blood. 2018; 131: 2602-2603.
25. Li Y, Xie J, Xu X. Inducible interleukin 32 (IL-32) exerts extensive antiviral function via selective stimulation of interferon λ1(IFN-λ1). J Biol Chem. 2013; 288.
26. Cai B, Cai J, Luo Y. The specific roles of JAK/STAT signaling pathway in sepsis. Inflammation. 2015; 38(4): 1599-1608.
27. Xiong W, Wang X, Liu X. Interferon-inducible MyD88 protein inhibits hepatitis B virus replication. Virology. 2004; 319: 306-314.