

Fc Gamma RIIb Expression Levels in Patients With Chronic Hepatitis B Virus Infection

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Research Article

Keywords: FcγRIIb, HBV infection, Chronic hepatitis B.

Posted Date: September 3rd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-757337/v1>

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Abstract

Background: Fc gamma receptor IIb (FcγRIIb), is an important inhibitory receptor which plays a vital role in regulating various immunoresponse processes and pathogenesis of many infectious disease. The purpose of our research is to show the FcγRIIb expression in serum and liver biopsy specimen of hepatitis B virus (HBV) suffers and explore its association with chronic HBV infection.

Methods: ELISA assay was adopted to identify the serum FcγRIIb levels in 119 HBV suffers and 24 healthy controls. The immunohistochemical method was then employed to identify FcγRIIb expression in the biopsy specimens of patients with chronic hepatitis B (CHB). The integrated optical density (IOD) value was measured to represent FcγRIIb expression levels.

Results: The serum FcγRIIb levels were decreased in CHB patients compared to controls ($P < 0.001$). FcγRIIb levels in CHB patient group were remarkably lower than in HBV carrier group ($P < 0.001$). In addition, the serum FcγRIIb levels were negatively associated with AST and ALT ($r = -0.3936$, $P = 0.0063$; $r = -0.3459$, $P = 0.0097$ respectively). The IOD values of FcγRIIb expression in moderate CHB and severity CHB group were significantly lower ($P = 0.006$, $P < 0.001$, respectively). The FcγRIIb level tended to be lower with the pathological changes of hepatitis. Further, the correlation analysis revealed FcγRIIb had negative correlations with AST and ALT ($r = -0.688$, $P = 0.0016$; $r = -0.686$, $P = 0.0017$ respectively) but positively associated with platelet counts ($r = 0.6464$, $P = 0.0038$).

Conclusions: FcγRIIb levels are significantly related to chronic HBV infection and progression of CHB. The change of FcγRIIb may affect the progression of liver inflammation and fibrosis in CHB patients

Background

Chronic hepatitis B virus (HBV) infection has always been a prime public health challenge across the world. According to the WHO global hepatitis report, the quantity number of HBV cases in 2015 was 257 million, and the infection rate was 3.5%[1]. Chronic HBV infection is a primary cause of liver cirrhosis and hepatocellular carcinoma (HCC). Moreover, it accounts for 786,000 HBV associated mortality annually, making it the tenth major cause of mortality death across the globe [2]. HBV can stimulate both innate and adaptive immunoresponse. The suppression of adaptive immunity is known as a crucial factor in maintaining HBV persistence infection through inhibition the killing effect of CD8 + T lymphatic cell [3, 4]. Innate immune response has been confirmed involved in viral clearance, and interferon, a key effector of innate immunity, have long been used to treat HBV infections[5].

FcγRIIb is the sole inhibitory FcγR, which can negatively modulate downstream signaling pathway[6]. It has three common isoforms, b1, b2 and b3, and b3 is the only soluble isoform that lacks of transmembrane domain and first cytoplasmic domain [7, 8]. FcγRIIb is expressed on the surface of nearly all leukocytes as well as B cell and involved in regulating these cell mediated immunity [9, 10]. Recent study shows that FcγRIIb also can be expressed in memory CD8 + T cells and regulate their activation and survival[11]. Another prominent feature of FcγRIIb is that it can be expressed on non-hematopoietic

cells, such as airway smooth muscle cells[12] or liver sinusoidal endothelial cells(LSECs)[13]. FcγRIIb expressed in LSECs is responsible for removal of small immunocomplex in hepatic sinusoids[14], which is essential to sustain liver immunity homeostasis. Ishikawa et al conducted a research on nonalcoholic fatty liver disease biopsy samples that progression of liver inflammation and fibrosis was associated with the declined expression of FcγRIIb in LSECs, and may influence its scavenger functions [15]. A study focused on HCC discovered that a decreased expression of FcγRIIb was more likely to have a higher cancer grade [16]. Moreover, fibrinogen like protein 2 (FGL2) is a member of the fibrinogen superfamily and secreted by the regulatory T (Treg) cells [17]. It has been reported capable of binding to FcγRIIb and exerting an immunosuppressive effect.[18].

FcγRIIb is a regulating molecule involved in various processes of both the innate and adaptive immune, such as antigen presentation, FcγR-mediated cellular activation, and apoptosis. It also regulates various signaling pathways such as TLR[19]and MAPK[20]. FcγRIIb is an important checkpoint of humoral tolerance in immune system of human. The hypofunction of FcγRIIb may result in activation of immunoresponse signaling and virus clearance [17]. Liver injury occurrence in HBV infection is strongly dependent on the host immune responses to the virus and is often accompanied by transaminases elevation. Thus FcγRIIb might be pivotal for immune reactions of the interaction between HBV and the host. Moreover, as a ligand of FcγRIIb, FGL2 participates in the immune response of persistent virus infections via regulating the FcγRIIb immune inhibitory pathway. Expression of FGL2 was associated with HBV infection and related to the clinical result of HBV associated liver diseases[21]. This study measured FcγRIIb expression levels in the HBV sufferers and analyzed the correlation with clinical parameters in attempt to elucidate the role of FcγRIIb in the immunoresponse process of chronic HBV infection.

Methods

Patients of serum samples

The serum samples come from 119 chronic HBV sufferers who have not taken any antiviral drugs, and 24 healthy individuals who attended physical health examination in the First Hospital of Jilin University between June 2019 and August 2020. According to the Guidelines for the Prevention and Treatment of Chronic Hepatitis B (2019 Edition) issued by the Chinese Medical Association, the patients were divided into HBV carriers and CHB patients. Patients who had other liver diseases, such as alcoholic hepatitis, fatty liver disease, acute or chronic infectious diseases, or autoimmune diseases were excluded. The basic and clinical data of patients and healthy controls were represented in Table 1. The study was approved by the research ethics committee of the First Hospital of Jilin University.

Patients of liver tissue samples

The liver tissue samples come from 12 patients with CHB and 4 controls with normal hepatic tissue who had undergone liver biopsy at the First Hospital of Jilin University between May 2015 and December 2018. Pathological diagnosis was defined according to the Guidelines for the Prevention and Treatment of Viral Hepatitis (2001) issued by the Chinese Medical Association. Fasting blood samples were

collected from patients before liver biopsy. The basic and clinical data of all patients were represented in Table 2. The study was approved by ethical review board of the First Hospital of Jilin University.

ELISA experiment

The ELISA experiments (soluble FcγRIIb: Jingmei, Jiangsu, China) were conducted according to manufacturers' protocols. Briefly, incubating the serum samples in 96-well plates coated with primary antibodies. After reaction, washing the plate and adding horseradish peroxidase labeled secondary antibodies into each well of the plate. And after reaction, washing again. Detecting protein mass by secondary antibody specific development reagents. Optical density of each well was identified via microplate reading device (Multiskan Sky, Thermo Fisher, USA).

Immunohistochemistry and morphometry

Immunohistochemical staining of FcγRIIb was performed on liver tissue specimens. The paraffin sections were de-waxed to water. Undergoing antigen unmasking with 10 mM citric buffering solution (PH6.0) for ten minutes at 95°C. Adding 3% hydrogen peroxide into each section to block the activity of endogenous peroxidase. After washing with PBS, incubating the sections with a primary rabbit anti-human FcγRIIb antiserum (Abcam, USA). After washing with PBS, then incubating the sections with enzymes second anti-rabbit antibody. Detecting with diaminobenzidine and then counterstaining with hematoxylin. The integrated optical density (IOD) values of the sample sections in every group were determined via Image-Pro Plus 6.0 software after tissue images program posterior to the collection of the sample images with a light microscopical device (× 40). Selecting 5 fields of vision stochastically to identify the positive IOD values, and then calculating the average IOD values, of which were deemed as the relative expression of FcγRIIb.

Basic biochemical parameters

Basic biochemical parameters were acquired by common approaches. Herein, our study analyzed assayed the sera concentration of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (γ-GTP), albumin (ALB) and HBV-DNA etc. The results of HBV-DNA were converted to log₁₀ IU/ml.

Statistics

All statistic assays were performed with GraphPad Prism 8. The experimental data are expressed as means ± standard deviation (SD). Comparisons of FcγRIIb expression levels were performed by the Kruskal-Wallis nonparametric test. Comparisons of IOD value of FcγRIIb expression were performed by one-way ANOVA test. Correlations were calculated using Spearman correlation analysis. A two-tailed p-value < 0.05 was of statistical significance.

Result

Serum expression levels of FcγRIIb in chronic HBV sufferers

Participate characteristics

Table 1 summarized the information of participates enrolled in this study including age, gender and clinical parameters. In total, most of participates were men. No differences existed between two groups regarding the age. In HBV carrier group, there were more patients with positive hepatitis B e antigen (HBeAg). In CHB group, more patients were HBeAg negative while anti- HBeAg were positive.

FcγRIIb expression level in serum

The values of serum FcγRIIb were 201.9 ± 15.19 ng/ml in healthy control group, 183.9 ± 33.47 ng/ml in HBV carrier group and 141.0 ± 37.14 ng/ml in CHB group. Compared with healthy control group, there was no significant difference of serum FcγRIIb level in HBV carrier group ($P = 0.123$), while FcγRIIb level in CHB patients was significantly lower ($P < 0.001$) (Figure. 1A). The serum FcγRIIb level in CHB patient group were significantly lower than in HBV carrier group ($P < 0.001$). Among all the HBV sufferers, the serum FcγRIIb level of positive HBeAg sufferers was statistically significantly lower than that of negative HBeAg sufferers ($P = 0.007$) (Fig. 1B).

Correlations with biochemical parameters

The correlations of FcγRIIb expression levels with sera biochemistry in CHB patients are shown in Fig. 2. FcγRIIb levels had a negative correlation with AST ($r = -0.3936, P = 0.0063$) and ALT ($r = -0.3459, P = 0.0097$). The regressive lines of sera ALP, γ-GTP and HBV DNA presented negative slopes, whereas no statistically significant association existed. The regressive line presented positive slopes for ALB, though there was no significant correlation.

Expression levels of FcγRIIb in tissue of CHB patients

Patient characteristics

Table 2 summarized the information of 18 participates enrolled in the study including age, gender and clinical parameters. With the biopsy proven pathologically inspection, five patients were confirmed as severity CHB, four patients were confirmed as mediate CHB and five patients as mild CHB. Four patients with normal liver tissue were control group.

3.2.2 FcγRIIb expression level in liver tissue

Typical optical microscope images of patients in different group are presented in Fig. 3. The FcγRIIb signals were discovered merely in LSECs[13]. In the study group, the IOD values of FcγRIIb were 23696.08 ± 3847.33 in control group, 21392.93 ± 7536.20 in mild CHB group, 10287.25 ± 2878.82 in mediate CHB

group and 5214.78 ± 1071.27 in severity CHB group. Compared with control group, no significant difference FcγRIIb level existed in mild CHB group, while FcγRIIb level in moderate CHB and severity CHB group were significantly lower ($P = 0.006$, $P < 0.001$, respectively). The FcγRIIb expression level in severity CHB and moderate CHB group were also lower than in mild CHB group ($P < 0.001$, $P = 0.018$, respectively). In other word, the FcγRIIb level tended to be lower with the pathological changes of hepatitis. (Fig. 4)

Correlation with biochemical parameters and pathological stage.

The correlations of FcγRIIb expression level of liver biopsy specimen with biochemical parameters and pathological stage are shown in Fig. 5. A correlation analysis showed that FcγRIIb had a negative relationship with AST ($r = -0.688$, $P = 0.0016$) and ALT ($r = -0.686$, $P = 0.0017$), and a positive relationship with platelets ($r = 0.6464$, $P = 0.0038$). The regressive lines of sera ALP, γ-GTP and HBV-DNA presented negative slopes, whereas no significant association existed. The regressive line presented positive slopes for ALB, though there was no significant correlation. The comparison of the pathological stage revealed that FcγRIIb had a significantly negative correlation with inflammation grade ($r = -0.913$, $P < 0.001$) and fibrosis state ($r = -0.875$, $P < 0.001$). It could be inferred that the decline of FcγRIIb levels in the development of chronic hepatitis is related to liver inflammation and fibrosis getting worse (Table 3).

Discussion

FcγRIIb is an inhibitory receptor, works as a regulation molecule of the immunosystem, and is vital for the progression of various autoimmune diseases and infectious diseases. In our previous study, we performed qRT-PCR assay to identify FcγRIIb mRNA expression in HBV sufferers[22]. The study showed that FcγRIIb expression was higher in the HBV carrier group in contrast to the CHB group. And we speculate that during the immune tolerance stage of HBV infection FcγRIIb expression played the role of immunosuppression. Thus declining in FcγRIIb expression in patients with CHB leads to a decreasing of the inhibition effect immune response and results in host immune activation. In this study, we discovered FcγRIIb levels of serum samples were significantly decreased compared to HBV carriers and healthy controls. It further suggested FcγRIIb could be a potential target of the immune response during chronic HBV infection.

During the early stage of virus infection, the innate immunity suppresses replicative activities and transmission, whereas the adaptive immunity mainly affects the virus removal during the late of infection period [23]. HBV can be recognized by related receptors and trigger antiviral innate immunity. Then the envelope antigen and nucleocapsid antigen of the virus can stimulate the adaptive immune response, with the help of CD4 + T cell, antibody can be produced and combined with HBV to form an immune complex that can be phagocytized by monocyte and macrophages. FcγRIIb can regulate expression and function of immune cells in innate immunity, such as maturation and antigen presentation ability of dendritic cells and the phagocytic ability of monocytes and macrophages[6]. A study has shown HBsAg-specific B cells in CHB patients exhibited a rised expression of suppressive receptors like PD-1 and FcγRIIb to suppress B cell activation and anti-viral immunity[24]. The clearance of persistent HBV

infection relies on the cytotoxic immune response of HBV-specificity CD8 + T cell. CD8 + T cells can induce cytotoxic reaction by recognizing antigen presented by APC and MHC-II antigen, which can promote apoptosis of HBV infected hepatocytes[25]. However, the persistent HBV infection may result from HBV-specific CD8 + T cells function impairment, in other words, the majority of HBV-specificity CD8 + T cells are able to activated but poorly proliferate and functionally exhausted[26, 27]. The mechanism of this functional decline is very complex, such as the expression of suppressive receptors and relevant ligands on hepatocytes increased, like PD-1 and PD-L1 [28, 29]. FcγRIIb can help dendrite cells acquire antigen from immune complexes and present antigen to Ag-specificity CD4 + and CD8 + T cell to induce robust immune reaction [24]. The vaccine chimegen HBV is a chimera immune therapy protein for treating chronic hepatitis B, and it is targeted dendritic cell. It could bind to immature dendrite cells and internalize FcγRII and mannose receptor (CD206) by endocytosis, which could lead to elevated MHC I and MHC II surface expression, induce T cell proliferation and restore HBV specific T cell function[30]. Treg cells are specific subgroup of CD4 + T cells which are crucial for constructing and sustaining immunotolerance. Tregs from CHB patients can suppress HBV specificity T cell immune response and result in a chronic, persistent HBV infection [31]. Moreover, FcγRIIb can bind to FGL2 working as an effector molecule of Treg cells and has immunoregulatory activity[32]. Hoang and his colleagues discovered that FGL2 levels were diseases remarkably increased in patients with cirrhosis and HCC in contrast to control group[21]. Therefore, FcγRIIb is vital for immune responses and is involved in the pathogenesis mechanism of virus infections, especially during HBV infection.

The levels of AST and ALT could denote the severe degree of liver damage during HBV infection, and the elevated liver enzyme can be viewed as a biomarker of immunity stimulation. In the early clinical studies, researchers found that patients with elevated ALT had a higher rate of HBV clearance than patients with normal ALT values. And the result highlights necessity of the activation of host immunosystem against virus antigens[33]. In our study FcγRIIb levels in CHB patient group were significantly lower than in HBV carrier group and had a negative relationship with ALT and AST. Immune tolerance occurs frequently during chronic HBV infection. Therefore, FcγRIIb may be a checkpoint to break the immune tolerance state, restore effector T cells, clear infected hepatocytes, and eliminate circulating immune antigen by humoral immunity.

It has been known that FcγRIIb is expressed on LSEC and that the liver is the major site of small immune complex including blood borne SIC clearance [13, 14]. FcγRIIb is a kind of scavenger receptor and the distributional feature of FcγRIIb has been presented in intrahepatic lobules of healthy livers [34]. Some researches revealed the association between FcγRIIb expressing change in LSECs and liver diseases. Ishikawa and his colleagues conducted a study on nonalcoholic liver disease biopsy samples to evaluate the FcγRIIb expressing on LSECs. The result showed an inverse proportion relationship between FcγRIIb expressing and fibrosis stages, and there are the highest expressing levels at the incipient stage of fibrosis and lowest at the third stage of fibrosis [15]. In patients with HCC, Geraud et al discovered a decreased expression of FcγRIIb was more likely to have a higher cancer grade. And FcγRIIb vanished in 63% in the adjacent tissue specimens of a microarray of HCC tissue [16]. Our study showed that in contrast to the controls, the expressing of FcγRIIb in moderate CHB and severe CHB group decreased

significantly. It also declined with the development of inflammation and fibrosis in CHB patients. Correlation analysis showed FcγRIIb expression levels on LSEs had a negative relationship with ALT and AST, which is consistent with serum results. Moreover, there was a positive relationship between FcγRIIb levels and platelet count. Platelets are remarkably related to liver inflammation and fibrosis. Gomez et al[35] discovered platelet count of patients with NASH decreased along with the development of liver fibrosis. And they reported platelet count could work as a new indirection marker of end-stage liver diseases and portal hypertension evaluation. Our study also showed a negative relationship between inflammation grade and fibrosis stage and FcγRIIb expressing levels via regression assay. In other words, patients with CHB that had a declined FcγRIIb expressing levels on LSEs may also have a lower platelet count, a higher inflammatory degree and a higher fibrosis phase. FcγRIIb may be related to the enhancement of liver inflammation and fibrosis in CHB patients. However, more researches are needed to evaluate the importance and to unveil the function of FcγRIIb in chronic HBV infection.

Conclusion

In summary, our research is supportive to the conclusion that FcγRIIb levels are significantly related to the chronic HBV infection and progression of CHB. The change of FcγRIIb may influence the progression of hepatic inflammation and fibrosis in CHB patients.

Abbreviations

HBV: Hepatitis B virus; CHB: Chronic hepatitis B; HC: Healthy control; HBeAg: Hepatitis B e antigen; FcγRIIb: Fc gamma receptor IIb.

Declarations

Acknowledgements

We thank all the patients and healthy individuals for their participation.

Funding

This study was funded by Jilin Province Science and Technology Development Plan Project (Grant No. 20190701053GH).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JN designed and supervised the studies. YL and XX conducted the experiments. JJ, YL and ZW evaluated the clinical data and provided the clinical samples. JJ and XX and ZW analyzed the data and wrote the manuscript. All authors read and approved the manuscript.

Ethics approval and consent to participate

The study was approved by the ethics committee of the First Hospital of Jilin University. All experiments were performed in accordance with relevant guidelines and regulations.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Due to technical limitations, table 1-3 is only available as a download in the Supplemental Files section.

Figures

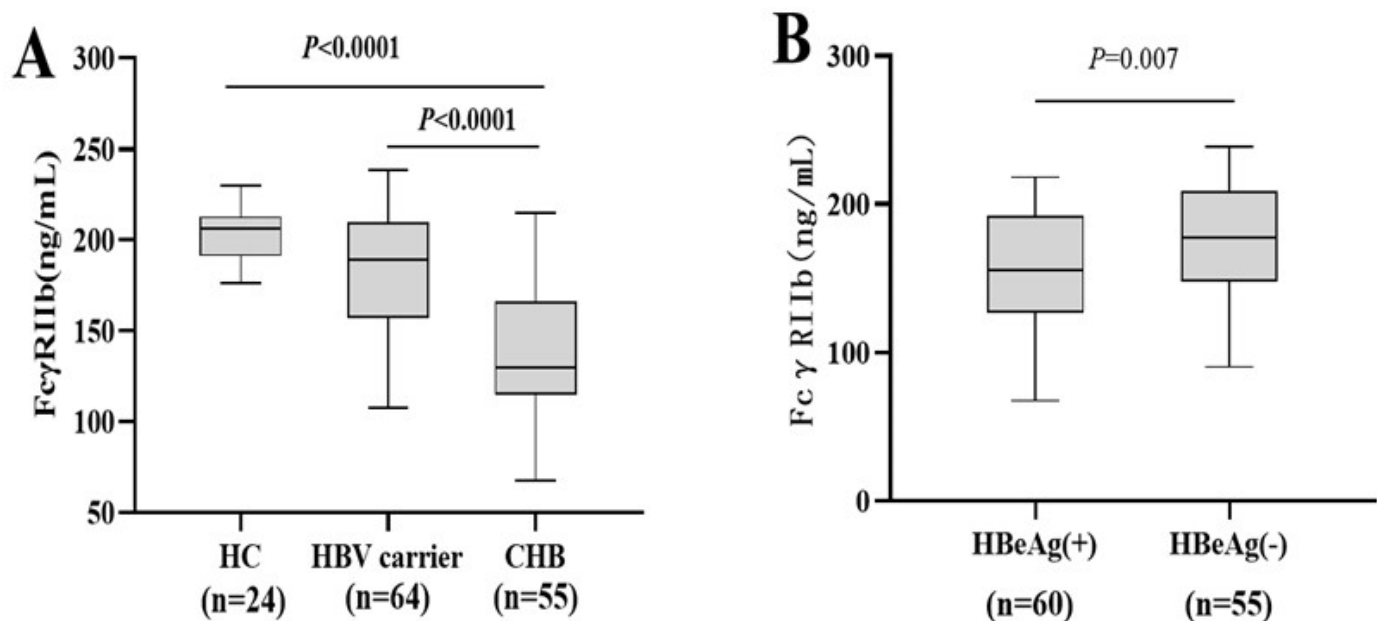


Figure 1

Fc γ RIIb levels in patients with chronic HBV infection and in healthy controls. A: Fc γ RIIb levels were measured in study subjects and compared between subgroups. B: comparison HBeAg positive group and HBeAg negative group. HC, healthy controls; CHB, chronic hepatitis B; Box plots illustrate medians with inter-quartile range. P values were calculated by Mann-Whitney-Wilcoxon test.

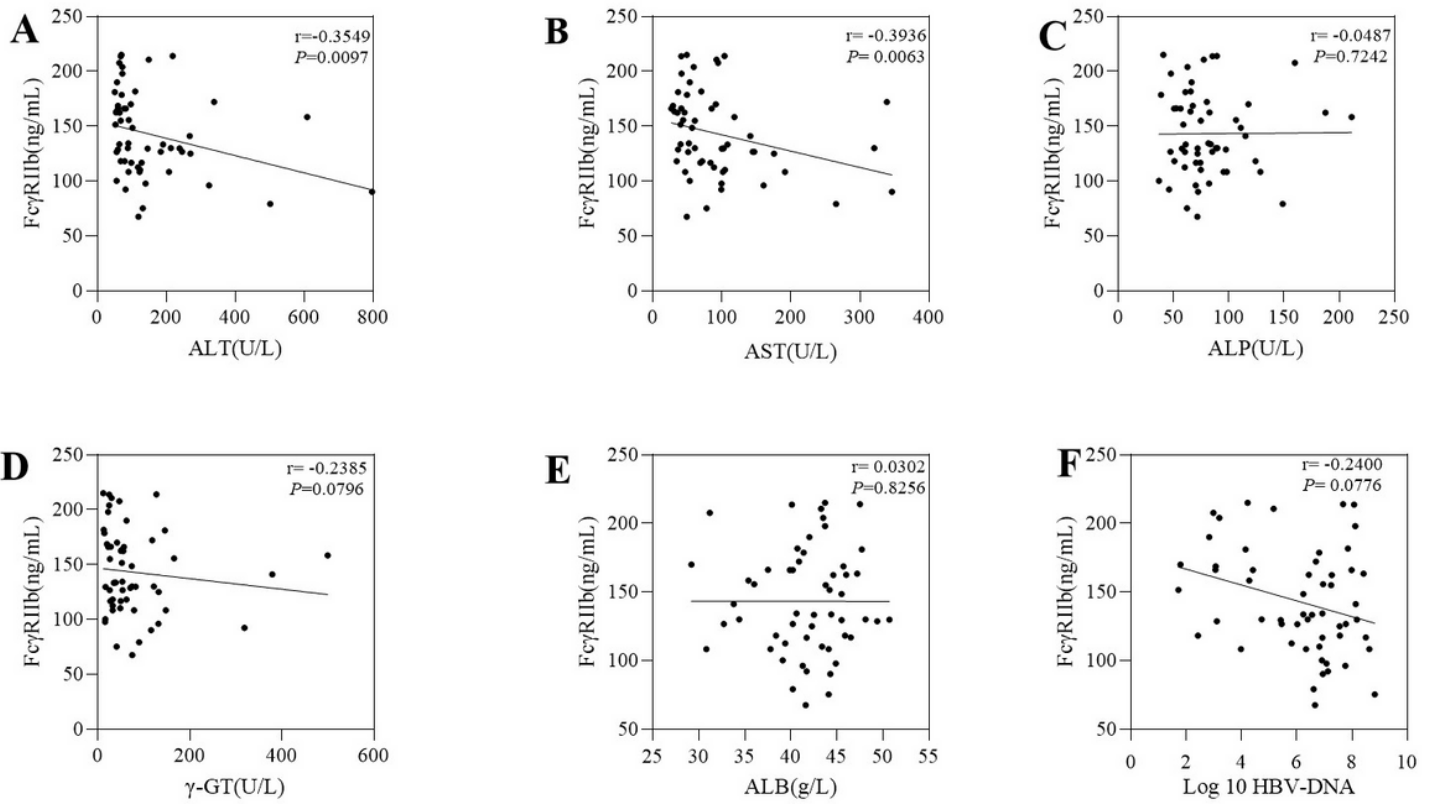


Figure 2

Correlation of FcγRIIb levels with clinical parameters of HBV infection. Correlations of FcγRIIb levels with different available clinical parameters were calculated by using Spearman's rank correlation coefficient test. The Spearman's rho and P value are also presented. A: between FcγRIIb levels and alanine amino transferase (ALT); B: between FcγRIIb levels and aspartate amino transferase (AST); C: between FcγRIIb levels and alkaline phosphatase; D: between FcγRIIb levels and γ -glutamyl transpeptidase (γ -GT) levels; E: between FcγRIIb levels and albumin levels; F: between FcγRIIb levels and HBV DNA load.

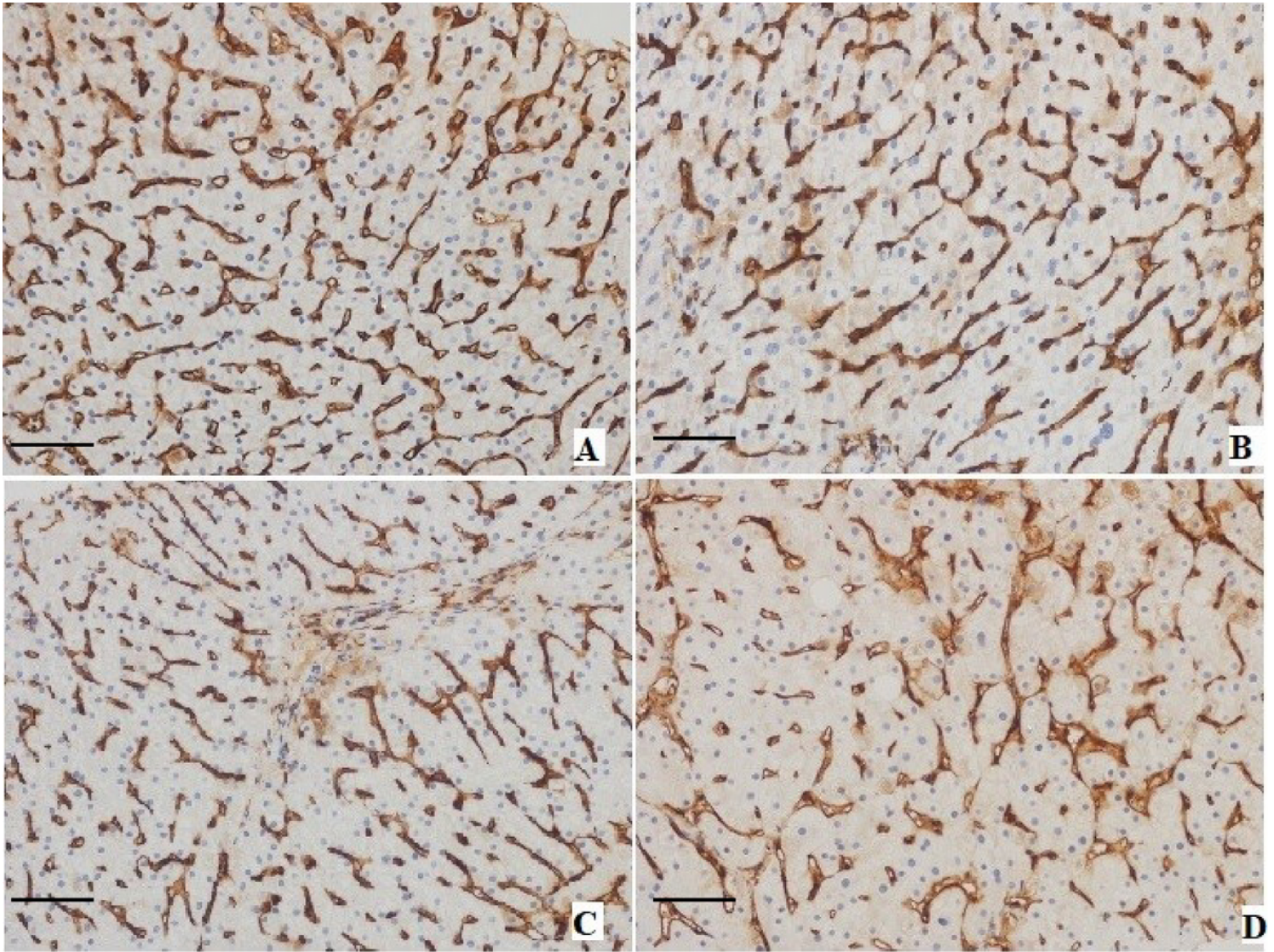


Figure 3

Representative light microscopic images of FcγRIIb expression in liver tissues of patients with chronic hepatitis B and controls (a: controls b: mild CHB c: moderate CHB d: severe CHB). FcγRIIb signals detected with DAB (brown) Nuclei were counterstained with hematoxylin (blue). Scale bars represent 100 μm.

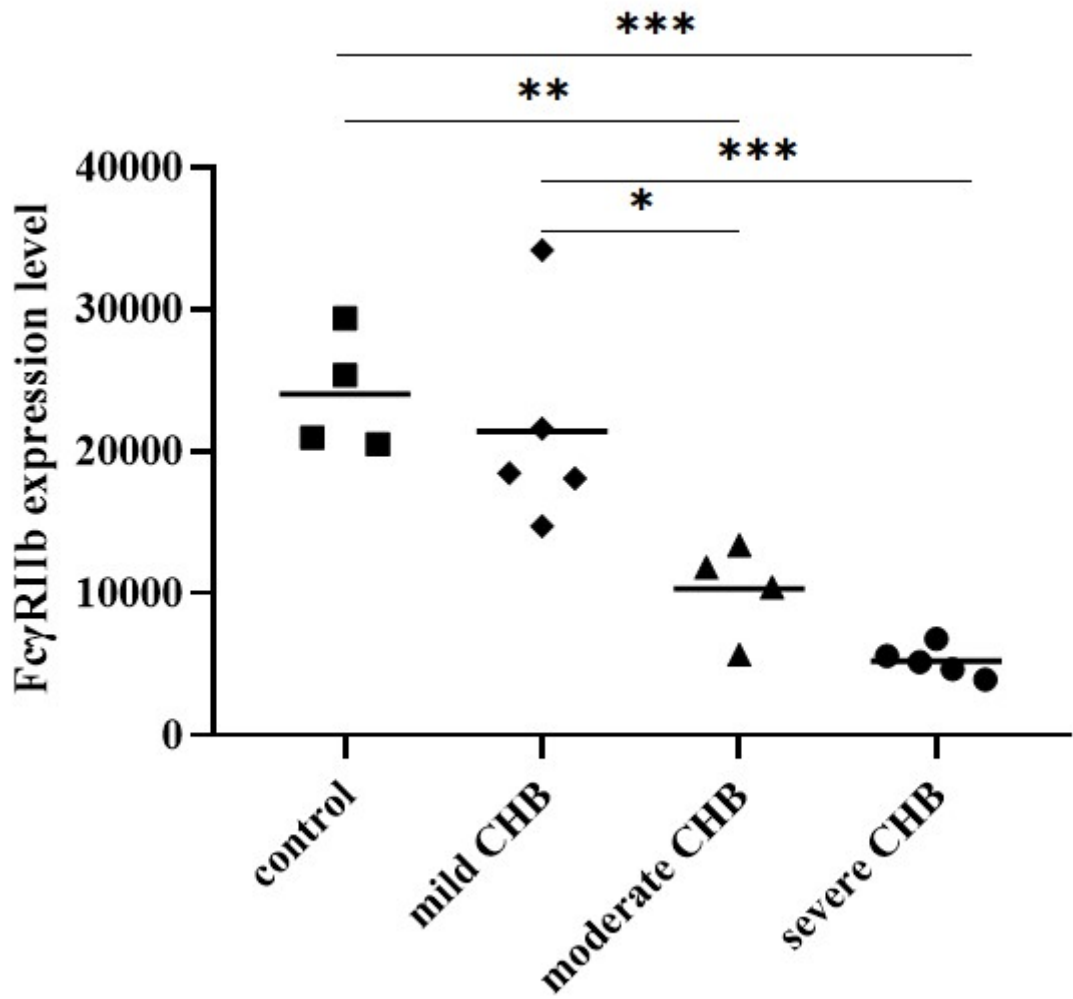


Figure 4

FcγRIIb levels in liver tissue of patients with chronic HBV infection and in healthy controls. P values were calculated by one-way ANOVA test.

Image not available with this version

Figure 5

The correlations of FcyRIIb expression level of liver biopsy specimen with biochemical parameters and pathological stage are shown in Fig 5.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table1.xlsx](#)
- [Table2.xlsx](#)
- [Table3.xlsx](#)