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Original Article

Accumulation of platelets in the liver may be an important contributory factor to liver injury in chronic hepatitis B virus infection

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Abstract: Background: Besides hemostatic properties, platelets have the features of inflammatory cells. Platelets have been shown to be contributed to the pathogenesis of acute liver damage of self-limited viral hepatitis and hepatitis B virus (HBV)-associated liver cancer in mouse models. However, the association between the platelets and chronic HBV infection remains largely unknown. We aimed to clarify whether an accumulation of platelets in the liver contributes to liver injury and fibrosis in chronic HBV infection. Methods: Sixty-five patients with chronic HBV infection who underwent liver biopsy were included. Twenty-three healthy liver tissue samples were obtained from donors whose livers were used for transplantation. The platelets in the liver tissues were identified by immunohistochemistry. According to the modified histology activity index described by Scheuer, the degree of hepatic inflammation and fibrosis of patients with chronic HBV infection was graded. Results: Patients with chronic HBV infection had a significantly more extensive CD61+ platelets in the liver tissues compared to healthy controls (69.08±6.21 vs. 16.68±2.05/HPF, P<0.001). Chronic HBV infected patients with higher inflammatory grading scores had more CD61+ platelets in their livers compared to those with lower scores (P<0.05). However, no association between liver platelets and fibrotic staging scores was found in the patients (r=0.069, P=0.586). The platelets in the liver tissues of patients with chronic HBV infection were also positively correlated with alanine transaminase (r=0.291, P=0.021), aspartate aminotransferase (r=0.328, P=0.009) and total bilirubin (r=0.236, P=0.036) levels. Conclusions: The accumulation of platelets in the liver may be involved in liver injury of patients with chronic HBV infection.

Keywords: Chronic hepatitis B, platelets, liver injury, liver fibrosis

Introduction

Despite the availability of effective vaccines and antiviral treatment modalities, chronic hepatitis B virus (HBV) infection is a widespread infectious disease with unfavorable outcomes and life-threatening consequences [1, 2]. Chronic HBV infection is the principal cause of liver cirrhosis and hepatocellular carcinoma (HCC) [1, 2]. Although, the pathogenesis of chronic HBV infection has been intensively studied, it is not well understood.

Platelets, upon activation, have been demonstrated to play a large role in initiation and perpetuation of inflammatory responses [3, 4]. Platelets may function to modulation of local inflammatory events through the release of chemokines, cytokines, growth factors, hemostatic proteins, and bactericidal agents [5, 6]. Platelets contribute to cytotoxic T lymphocyte (CTL)-mediated liver immunopathology in mouse models of acute viral hepatitis independent of procoagulant function and platelet depletion may reduce the intrahepatic accumulation of virus-specific CTLs and organ damage [7, 8].

Recent studies have demonstrated that antiplatelet therapy suppresses hepatic immunopathology and prevents or delays the development of HCC, as well as improving survival in

Table 1. Clinical features of the patients with chronic HBV infection

	CHB (n=65)
Ages (yr)	48.22±10.27
Gender (M/F)	40/25
Hb (g/L)	108.78±24.59
PLT (×10^9/L)	67.10±43.80
ALT (U/L)	46.53±57.52
AST (U/L)	53.70±76.46
ALP (U/L)	80.53±32.06
GGT (U/L)	47.68±46.245
Tbil (μmol/L)	35.57±37.75
Alb (g/L)	35.85±4.81
HBeAg positive (%)	16 (24.61)
HBV DNA (IU/ml)	3.79±1.34
Child-pugh scores	6.43±1.42
Inflammatory grades	
G0/G1/G2/G3/G4	1/17/34/10/3
Fibrosis stages	
S0/S1/S2/S3/S4	3/7/8/16/31

Alb, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CHB, chronic hepatitis B; GGT, gamma-glutamyl transferase; Hb, hemoglobin; G, inflammatory grades; S, fibrotic stages; PLT, platelets; Tbil, total bilirubin.

mouse model of chronic HBV infection [9]. Kondo et al investigated the platelets in the liver tissues of chronic hepatitis C (CHC) patients and they found that accumulation of platelets may involve in thrombocytopenia and liver fibrosis through the activation of hepatic stellate cells (HSCs) in CHC [10].

However, to the best of our knowledge, the platelet kinetics in the liver tissues of patients with chronic HBV infection is not fully elucidated. Therefore, the aim of the present study was to clarify the histopathological findings of platelets in human liver tissues of patients with chronic HBV infection.

Patients and methods

Study subjects

Sixty-five patients with chronic HBV infection who underwent liver biopsy are included. Twenty-three specimens of healthy liver tissues obtained from donors whose livers were used for transplantation were used as controls. The study was performed with informed consent obtained from patients for the use of their liver tissues in the investigation, and was approved by the ethical committee. The basic characteristics of the enrolled subjects are listed in **Table 1**.

Histopathology

The liver tissues were fixed with 10% formalin and embedded in paraffin. The tissues were cut into 2 μm sections and then used for histological and immunohistological analyses. The sections were stained with hematoxylin and eosin and examined under a light microscope. The degree of hepatic inflammation and fibrosis of liver fibrosis was evaluated according to the classification proposed by Scheuer [11]. The histopathological diagnosis and classification was performed by the experienced pathologist.

Immunohistochemistry

Immunohistochemical staining was performed on 2 μm paraffin sections of tissues. Sections were deparaffinized and blocked endogenous peroxidase with 3% H_2O_2. The antigen retrieval was performed using a standard protocol. A mouse anti-human CD61 monoclonal antibody (Dako, Glostrup, Denmark) was used as the primary antibody. CD61 is a platelet-specific antigen. The slides were incubated with primary antibodies overnight at 4°C and then MA2000. MA2000 polymer immunohistochemical detection system at 37°C for 20 min (Beijing Zhong Shan Jinqiao Biological Technology Co., Ltd, China). Stained sections were developed with diaminobenzidine (Life Technologies, Carlsbad, USA) and counterstained with hematoxylin (Dako, Glostrup, Denmark).

Measurement of cells

Five sections of each sample were randomly selected and photomicrographs were obtained at a magnification of 400× and captured for analysis using Image Pro-Plus 5.0 software (Media Cybernetics, SilverSpring, MD, USA). The numbers of CD61+ positive cells per high-power field (HPF) were counted in a blinded fashion by the experienced pathologist [12].

Statistical analysis

All data analysis was performed using SPSS 22.0 software (SPSS Inc., Chicago, IL, United

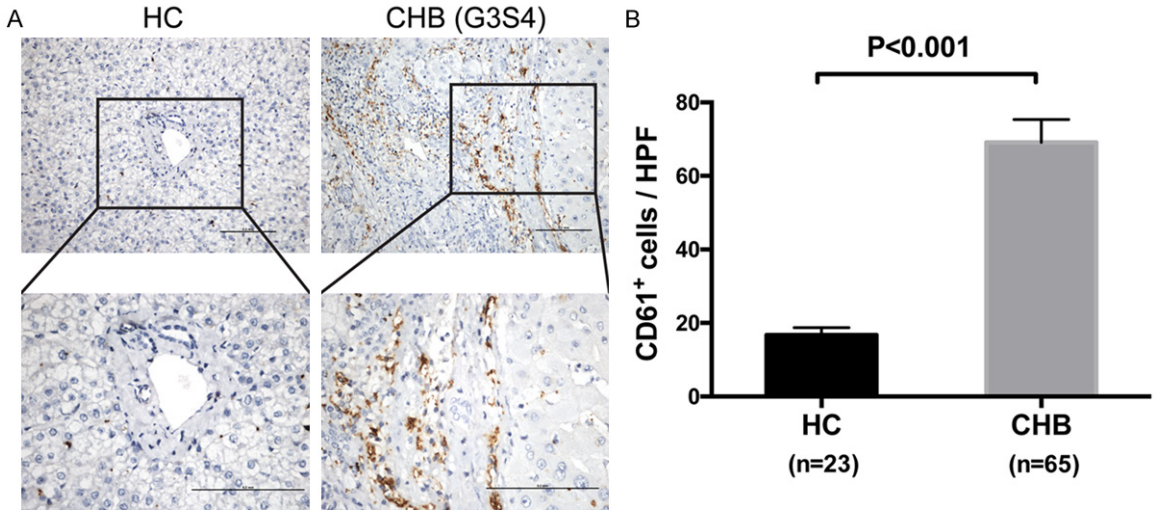

Figure 1. Immunohistochemical analysis of CD61+ platelet accumulation in the liver tissues of patients with chronic HBV infection. Representative images of CD61 by imunohistochemistry in the liver tissues of healthy control and a patient with chronic hepatitis B (A). Comparison of CD61+ platelets between healthy controls and patients with chronic HBV infection (B). CHB, chronic hepatitis B; G, inflammatory grades; HC, healthy control; HPF, high-power field; S, fibrotic stages. Scale bars represent 200 μm.

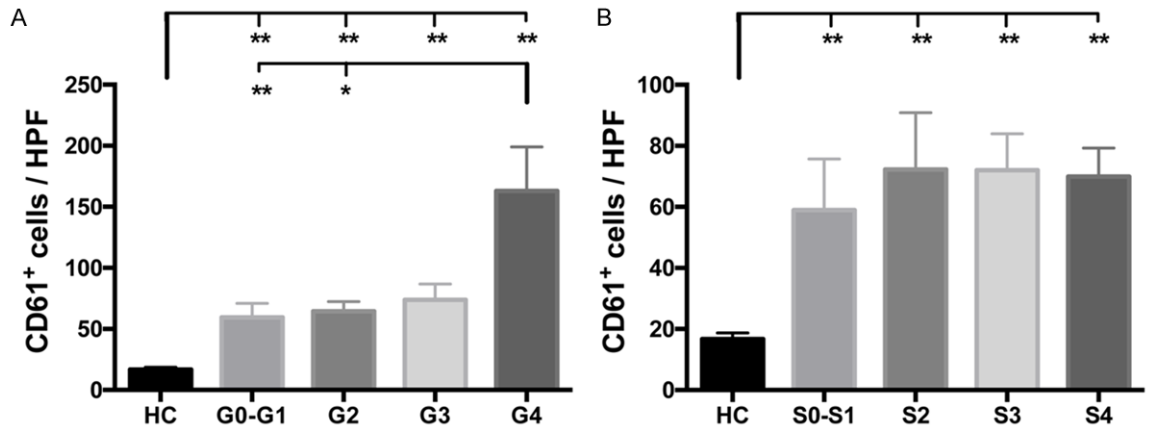

Figure 2. Comparison of CD61+ platelets in chronic HBV infected patients with different inflammatory grades (A) and fibrotic stages (B). G, inflammatory grades; S, fibrotic stages. *P<0.05, **P<0.01.

States). Continuous variables were expressed as mean and standard deviation (SD) or standard error of the mean (SEM). Statistical comparisons between two groups were performed by Mann-Whitney-U-test, multiple comparisons by ANOVA-test or Kruskal-Wallis H test. Correlation analysis was performed by Pearson or Spearman rank correlation test. A *P*-value <0.05 was considered statistically significant.

Results

Patient characteristics

Sixty-five patients with chronic HBV infection were included in the present study. The baseline demographics are shown in **Table 1**. The mean age of patients was 48.22±10.27 years and 40 (61.53%) of the patients were male. Sixteen (24.61%) patients were HBeAg positive.

Intrahepatic accumulation of platelets in patients with chronic HBV infection

In the normal control group, there was a small amount of positive CD61 staining cells. The density of CD61+ platelets was significantly increased in the livers of patients with chronic HBV infection (69.08±6.21/HPF) as compared with normal tissues (16.68±2.05/HPF, P<

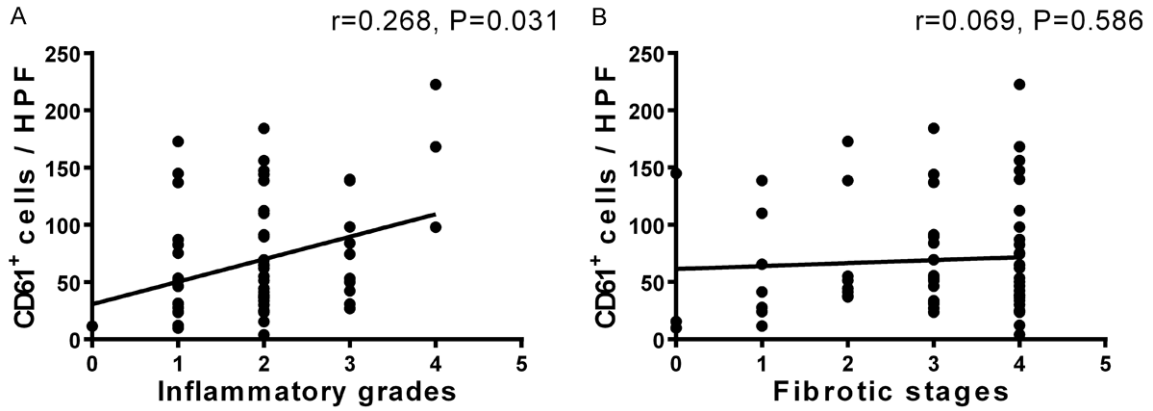

Figure 3. Correlations between the numbers of CD61+ platelets in chronic HBV infected patients with inflammatory grades (A) and fibrotic stages (B).

Table 2. The clinical relevance of CD61+ platelets in patients with chronic HBV infection

	r	*P* value
Hb	0.159	0.214
PLT	0.042	0.746
ALT	0.291	0.021
AST	0.328	0.009
ALP	-0.007	0.957
GGT	0.065	0.611
Tbil	0.236	0.038
Alb	-0.016	0.898
HBV DNA	-0.120	0.964
Child-pugh scores	0.006	0.964

Alb, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; Hb, hemoglobin; PLT, platelets; Tbil, total bilirubin.

0.001). The platelets were predominantly seen in the sinusoidal space of the periportal area with inflammation in the liver tissues of patients with chronic HBV infection (**Figure 1**).

Intrahepatic accumulation of CD61+ platelets correlates with inflammatory grades and fibrotic stages in patients with chronic HBV infection

Patients with higher inflammatory grading (G) scores had significantly more CD61+ platelets in their livers compared to those with lower scores (**Figure 2**). Significantly more CD61+ platelets were found in the liver tissues of patients with different fibrotic staging (S) scores compared to normal control group (**Figure 2**). The density of CD61+ platelets was positively correlated with G scores (r=0.268, P=0.031), but not correlated with S scores (r=0.069, P=0.586) (**Figure 3**).

The clinical relevance of CD61+ platelets

The association between the CD61+ platelets and the clinical parameters were analyzed (**Table 2**). CD61+ platelets numbers in the liver tissues of patients with chronic HBV infection were positively correlated with serum alanine transaminase (ALT) (r=0.291, P=0.021), aspartate aminotransferase (AST) (r=0.328, P= 0.009) and total bilirubin (r=0.236, P=0.036) levels.

Discussion

A growing body of evidence derived from rodent and in vitro studies highlights the role for platelets as active players in liver inflammation and liver fibrosis [13, 14]. In chronic HCV infection, patients with HCV related liver cirrhosis had a more extensive platelet area in the liver compared to controls [10]. However, the role of platelets in the pathogenesis of chronic HBV infection is not clear. In the present study, we investigated the platelets in the liver tissues of patients with chronic HBV infection. We found that the platelets were significantly accumulated in the liver tissues of patients with chronic HBV infection and platelets were mainly clustered in the portal areas or within the lobule. Furthermore, the numbers of platelets were correlated with inflammatory grades as well as ALT and AST levels, which indicating that plate-

lets may be involved in inflammatory reactions for destruction of infected cells.

Platelets can express a variety of adhesion molecules and immune receptors on their membranes which are instrumental for interactions with the endothelium and different subsets of circulating leukocytes to achieve their immunological functions [15, 16]. In the injured liver, platelets may interact with hepatic sinusoidal endothelial cells (HSECs) and influence effector cell recruitment and activation [13]. In vitro study has demonstrated that platelets may activate isolated HSECs to express chemokine (C-X-C motif) ligand 8 and chemokine (C-C motif) ligand 2, thereby promoting neutrophil and lymphocyte recruitment [17]. Furthermore, platelets also interact with neutrophils to trap microbes in neutrophil extracellular traps, which may promote neutrophil mediated hepatotoxicity [18]. In vivo murine experiments, platelets are a primary mediator of viral hepatitis [13]. Platelets are present at sites of tissue damage in the mouse models of CD8+ T cell-mediated acute viral hepatitis and depletion of the platelets may ameliorate disease severity by reducing the accumulation of hepatic virus-specific CD8+ T cells [7]. Antiplatelet therapy can effectively diminish the number of intrahepatic HBV-specific CD8+ T cells and HBV-nonspecific inflammatory cells, and can prevent the development of HCC prevent in a mouse model of chronic immune-mediated hepatitis B [9]. However, the mechanisms of platelets in the pathogenesis of liver injury of chronic HBV infection deserve further investigation.

We note that the platelet infiltration is not correlated with the stage of fibrosis in chronic HBV infected patients in the present study. However, platelets have been reported to play a role in liver fibrosis in mice [13]. Rodent studies reveal that platelet lysates can drive the profibrotic cytokine secretion by HSCs in vitro and exacerbate fibrosis [13]. However, platelets may also suppress fibrogenesis and initiate fibrolytic pathways. Platelets can reduce liver fibrosis and promote liver regeneration in mice by secreting hepatocyte growth factor [19]. Although the platelet area in liver tissues increased along with an increase in fibrosis stages in patients with CHC, all the liver tissues were obtained from the patients who underwent hepatectomy for HCC with hepatitis C virus infection and the platelets were detected in the noncancerous liver tissues [10]. Thus, more studies are needed to explore the contribution of platelets to liver fibrosis in chronic HBV infection.

In conclusion, the present study indicated that the accumulation of platelets in the liver may be involved in liver injury of patients with chronic HBV infection. The study provides additional evidence on the important role of platelet in the pathogenesis of chronic HBV infection. However, further studies of the biological functions of infiltrating platelets in the liver tissues of patients with chronic HBV infection are needed.

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Disclosure of conflict of interest

None.

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References

[1] Trépo C, Chan HL, Lok A. Hepatitis B virus infection. Lancet 2014; 384: 2053-2063.
[2] Liaw YF, Chu CM. Hepatitis B virus infection. Lancet 2009; 373: 582-592.
[3] Klinger MH. Platelets and inflammation. Anat Embryol (Berl) 1997; 196: 1-11.
[4] Yeaman MR. The role of platelets in antimicrobial host defense. Clin Infect Dis 1997; 25: 951-68; quiz 969-70.

[5] Elzey BD, Tian J, Jensen RJ, Swanson AK, Lees JR, Lentz SR, Stein CS, Nieswandt B, Wang Y, Davidson BL, Ratliff TL. Platelet-mediated modulation of adaptive immunity. A communication link between innate and adaptive immune compartments. Immunity 2003; 19: 9-19.

[6] Matowicka-Karna J, Kamocki Z, Polińska B, Osada J, Kemona H. Platelets and inflammatory markers in patients with gastric cancer. Clin Dev Immunol 2013; 2013: 401623.

[7] Iannacone M, Sitia G, Isogawa M, Marchese P, Castro MG, Lowenstein PR, Chisari FV, Ruggeri ZM, Guidotti LG. Platelets mediate cytotoxic T lymphocyte-induced liver damage. Nat Med 2005; 11: 1167-1169.

[8] Morrell CN, Aggrey AA, Chapman LM, Modjeski KL. Emerging roles for platelets as immune and inflammatory cells. Blood 2014; 123: 2759-2767.

[9] Sitia G, Aiolfi R, Di Lucia P, Mainetti M, Fiocchi A, Mingozzi F, Esposito A, Ruggeri ZM, Chisari FV, Iannacone M, Guidotti LG. Antiplatelet therapy prevents hepatocellular carcinoma and improves survival in a mouse model of chronic hepatitis B. Proc Natl Acad Sci U S A 2012; 109: E2165-2172.

[10] Kondo R, Yano H, Nakashima O, Tanikawa K, Nomura Y, Kage M. Accumulation of platelets in the liver may be an important contributory factor to thrombocytopenia and liver fibrosis in chronic hepatitis C. J Gastroenterol 2013; 48: 526-534.

[11] Scheuer PJ. Classification of chronic viral hepatitis: a need for reassessment. J Hepatol 1991; 13: 372-374.

[12] Cao D, Xu H, Guo G, Ruan Z, Fei L, Xie Z, Wu Y, Chen Y. Intrahepatic expression of programmed death-1 and its ligands in patients with HBV-related acute-on-chronic liver failure. Inflammation 2013; 36: 110-120.

[13] Chauhan A, Adams DH, Watson SP, Lalor PF. Platelets: no longer bystanders in liver disease. Hepatology 2016; 64: 1774-1784.

[14] Ripoche J. Blood platelets and inflammation: their relationship with liver and digestive diseases. Clin Res Hepatol Gastroenterol 2011; 35: 353-357.

[15] Elzey BD, Sprague DL, Ratliff TL. The emerging role of platelets in adaptive immunity. Cell Immunol 2005; 238: 1-9.

[16] Jenne CN, Urrutia R, Kubes P. Platelets: bridging hemostasis, inflammation, and immunity. Int J Lab Hematol 2013; 35: 254-261.

[17] Lalor PF, Herbert J, Bicknell R, Adams DH. Hepatic sinusoidal endothelium avidly binds platelets in an integrin-dependent manner, leading to platelet and endothelial activation and leukocyte recruitment. Am J Physiol Gastrointest Liver Physiol 2013; 304: G469-478.

[18] Ma AC, Kubes P. Platelets, neutrophils, and neutrophil extracellular traps (NETs) in sepsis. J Thromb Haemost 2008; 6: 415-420.

[19] Watanabe M, Murata S, Hashimoto I, Nakano Y, Ikeda O, Aoyagi Y, Matsuo R, Fukunaga K, Yasue H, Ohkohchi N. Platelets contribute to the reduction of liver fibrosis in mice. J Gastroenterol Hepatol 2009; 24: 78-89.