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Original Article

Beta2-Glycoprotein I in Hepatitis B virus infection patients with hepatocellular carcinoma, chronic hepatitis B and acute hepatitis B

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Abstract: This study aimed to evaluate the role of plasma Beta 2-Glycoprotein I (β2GPI) on hepatitis B virus (HBV) infection patients with hepatocellular carcinoma (HCC), chronic hepatitis B (CHB) and acute hepatitis B (AHB). A total of 12 healthy controls (HC) and 107 HBV infection patients, including 43 HCC, 54 CHB and 10 AHB were enrolled in this study. The plasma concentrations of β2GPI were determined by enzyme-linked immunosorbent assay (ELISA), and serum concentrations of TH1 and TH2 type cytokines were determined by cytometric bead array (CBA). Correlations between β2GPI and clinicopathologic data were evaluated by Spearman rank correlation test, and the diagnostic values of β2GPI were analyzed by receiver operating characteristic (ROC) curves. The plasma level of β2GPI was significantly elevated in HBV infection patients with HCC and CHB than HC ($P < 0.05$), while no significant difference was revealed between AHB and HC. Combination of α-fetoprotein (AFP) and β2GPI could significantly improve the diagnosis performance of HBV-related HCC (AUC, 0.887). Besides, β2GPI was associated with HbeAg in CHB patients, and a positive correlation was revealed on β2GPI and IFN-γ in AHB patients. Plasma β2GPI was closely associated with HBV-related HCC, which could be used as a novel diagnosis marker in clinic.

Keywords: Hepatitis B virus, Beta 2-Glycoprotein I, hepatocellular carcinoma, chronic hepatitis B, acute hepatitis B

Introduction

Hepatitis B virus (HBV) is a small DNA virus belongs to hepadnaviridae family [1]. Its infection on human has become one of the major global health problems, which exhibits a prevalence ranges from over 10% in Asia to under 0.5% in the United States and Northern Europe [2, 3]. In clinic, chronic hepatitis B (CHB) is always associated with a high risk of hepatocellular carcinoma (HCC), which lead to an annually of nearly 1 million death in the world [4]. The increased morbidity and mortality of HCC have attracted our interest on prognosis of HBV-related HCC. Recently, the most commonly used biological marker for HCC is serum level of α-fetoprotein (AFP) [5]. However, the use of AFP on HCC, especially on HBV-related HCC is still limited by low diagnostic accuracy and specificity [6, 7]. Therefore, there is an urgent need of improved indicators for early and noninvasive diagnosis of HBV-related HCC.

Beta 2-Glycoprotein I (β2GPI), formerly known as apolipoprotein H, is a 50 kDa phospholipid-binding plasma protein primarily synthesized in liver [8]. *In vivo*, β2GPI is always involved in coagulation and apoptotic processes by binging to anionic phospholipids [9]. Meanwhile, it is also an autoantigen in patients with antiphospholipid antibodies, and considered to be important in HBV infection [10]. It has been reported β2GPI could bind to the surface antigen of HBV, and the binding activity was higher in sera from patients in active virus replication phase [11]; High expression of β2GPI could enhance the binding of HBsAg to cell surfaces, thus contributed to virus particle transfer to sodium-taurocholate co-transporting polypeptide (NTCP) receptor and interaction with annexin II for viral

Table 1. The basic characteristics of hepatitis B virus (HBV) infection patients

Variables	HCC	CHB	AHB	HC
Sample size	43	54	10	12
Age (years)	56.51 ± 7.52	43.98 ± 11.73	38.90 ± 10.13	43.33 ± 14.87
Gender (M/F)	34/9	37/17	8/2	8/4

HCC, hepatocellular carcinoma; CHB, chronic hepatitis B; AHB, acute hepatitis B; HC, healthy controls.

membrane fusion [12]. Furthermore, an important role of β2GPI on the development of HBV-related HCC is also revealed. As reported, NF-κB could be activated by interaction of β2GPI and HbsAg [13]; lipopolysaccharide enhanced signal transduction of β2GPI in HCC cells could lead to the activation of NF-κB, and thus promoted the development of HCC [14]. However, whether β2GPI could be used as an indicator for HBV-related HCC, and its relations with CHB and acute hepatitis B (AHB) were still unclear.

In this study, the plasma level of β2GPI was detected in HBV infection patients and its correlations with HCC, CHB and AHB were further analyzed. Our findings may reveal the potentially indicative role of β2GPI in HBV-related liver diseases, which would be a benefit to the early and noninvasive diagnosis of HBV-related HCC.

Materials and methods

Subjects

A total of 107 HBV infection patients, including 43 HCC, 54 CHB and 10 AHB were recruited form the First Hospital of Jilin University, China between January 2013 and August 2014. HCC, CHB and AHB were diagnosed by American Association for the Study of Liver Diseases (AASLD) practice guideline [15, 16] HCC and liver cirrhosis were further identified by computed tomography (CT), magnetic resonance imaging or biopsy results. Besides, 12 healthy volunteers without HBV and autoimmune liver diseases were used as control group (HC) (**Table 1**). This study was approved by Human Ethics Committee of Jilin University, and written informed consents were obtained from all subjects.

Measurement of plasma β2GPI by enzyme-linked immunosorbent assay (ELISA)

Plasma concentrations of β2GPI were determined by a human β2GPI ELISA kit (Cloud-Clone Corp, USA). Simply, plasma samples were firstly isolated from peripheral blood of all subjects, and incubated with anti-β2GPI for 2 hours at 37°C. Then the samples were washed with PBS, and peroxidase-labeled biotinylated secondary antibodies were added. After induction for 1 hour at 37°C, the samples were treated with 3,3',5,5'-tetramethylbenzidine (TMB) substrate solution, and the reaction was stopped by TMB stop solution. Finally, optical density (OD) was measured at 450 nm by a microplate reader (Thermo Fisher Scientific, Finland).

Measurement of serum TH1 and TH2 type cytokines by Cytometric bead array (CBA)

Serum concentrations of TH1 and TH2 type cytokines, including IFN-γ, TNF-α, IL-2, IL-4, IL-17A, IL-10, and IL-6 were determined by CBA (BD Biosciences, USA) according to the manufacturer's protocol. A total of 25 μl serum samples were isolated from peripheral blood of all subjects, and then the concentrations of various cytokines were quantified by CellQuestPro and CBA software (Becton Dickinson, USA) on a FACSAria II (BD Biosciences, USA).

Statistical analysis

Statistical analysis was performed by SPSS version 18.0 (SPSS Inc., Chicago, IL). Continuous variables were expressed as mean ± standard deviation (SD). Comparisons between independent groups and multiple groups were analyzed by Mann-Whitney U test and Kruskal-Wallis test, respectively. Categorical variables were expressed as counts, and comparisons were assessed by Fisher exact test. Correlations between β2GPI and clinicopathologic data were evaluated by Spearman rank correlation test, and the diagnostic values of β2GPI were analyzed by receiver operating characteristic (ROC) curve. A *p*-value less than 0.05 was considered to be significantly different.

Results

The plasma level of β2GPI was elevated in patients with HBV-related HCC

As to evaluate the potentially indicative role of β2GPI in HBV-related liver diseases, the plas-

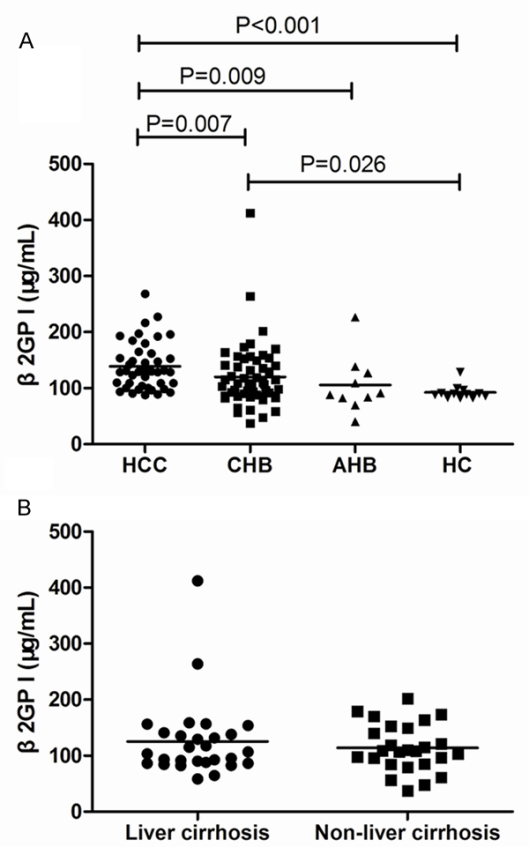

Figure 1. A. The plasma level of β2GPI in hepatitis B virus (HBV) infection patients with hepatocellular carcinoma (HCC), chronic hepatitis B (CHB), and acute hepatitis B (AHB). Healthy subjects were used as control (HC). B. The plasma level of β2GPI in CHB patients with or without liver cirrhosis.

ma level of β2GPI in HCC, CHB and AHB patients was detected. As shown in **Figure 1A**, the content of β2GPI was highest in patients with HCC (138.91 ± 42.07 μg/ml) ($P < 0.01$), followed by CHB (120.15 ± 57.83 μg/ml) and AHB (105.65 ± 50.85 μg/ml). In addition, a significantly higher β2GPI level was exhibited on HCC and CHB patients than HC (92.22 ± 12.65 μg/ml) ($P < 0.05$). Besides, the relation between β2GPI and liver cirrhosis was also evaluated in CHB patients, and no significantly differences were found in patients with or without liver cirrhosis (**Figure 1B**).

β2GPI improve the diagnosis performance of HBV-related HCC

Firstly, a cut-off of 200 ng/mL AFP was used to evaluate its correlation with β2GPI. As shown in **Figure 2A**, the plasma level of β2GPI in HBV

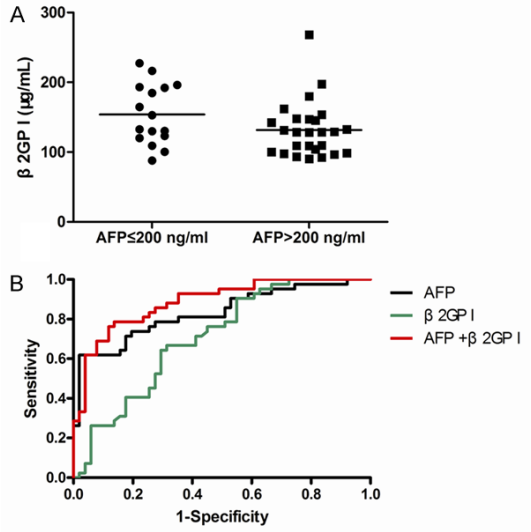

Figure 2. A. The plasma level of β2GPI in hepatitis B virus (HBV) related hepatocellular carcinoma (HCC) patients with low and high level ofα-fetoprotein (AFP). B. Receiver operating characteristic (ROC) curves of β2GPI (green), α-fetoprotein (AFP, black) and β2GPI combined with AFP (red) in diagnosis of HBV-related HCC.

infection patients with HCC was not significantly changed with high and low concentration of AFP (153.79 ± 43.24 μg/ml vs. 131.68 ± 39.46 μg/ml). Then the diagnosis performance of AFP and β2GPI on HBV-related HCC was further analyzed by ROC curve. As a result, the AUC of AFP and β2GPI was 0.832 (95% CI, 0.747-0.917) and 0.708 (95% CI, 0.604-0.812), respectively. To pay attention to, combination of AFP and B2GPI could significantly improve the diagnosis accuracy of HBV-related HCC (AUC, 0.887) (**Figure 2B**).

Correlations between β2GPI and clinical characteristics of HBV infection patients with HCC, CHB and AHB

Correlations between the plasma level of β2GPI and clinical characteristics of HBV-related HCC, CHB and AHB were also evaluated. As shown in **Table 2**, no significantly correlations were revealed on all enrolled clinical factors in patients with HCC, including gender, HBV DNA load, the level of ALT and AST, Child-Pugh stage, positive of HbeAg, alcohol drinking, tumor size and BCLC stage. In addition, significantly correlations were also not found in AHB patients (data was not shown). However, the plasma level of β2GPI was significantly increased in HBeAg-

Table 2. Correlations between the plasma level of β2GPI and clinical characteristics of hepatitis B virus (HBV) infection patients with hepatocellular carcinoma (HCC)

Variables (n[a])	β2GPI (μg/ml)	*P* value
Gender		0.709
Male (34)	136.30 ± 39.22	
Female (9)	148.79 ± 53.00	
HBV DNA load (log10 IU/mL)	-	0.405
ALT level (U/L)	-	0.842
AST level (U/L)	-	0.374
Child-Pugh stage		0.650
A (15)	147.97 ± 51.89	
B (19)	135.63 ± 35.59	
C (9)	130.76 ± 38.44	
HBeAg		0.619
Positive (9)	131.72 ± 9.26	
Negative (21)	139.76 ± 53.23	
Alcohol drinking		0.508
Yes (16)	134.52 ± 45.09	
No (24)	139.54 ± 42.42	
Tumor size		0.278
< 5 cm (7)	151.88 ± 45.88	
≥ 5 cm or multiple tumor (36)	136.39 ± 41.51	
BCLC stage		0.667
0 (1)	152.78	
A (6)	151.73 ± 50.26	
B (5)	143.90 ± 45.89	
C (21)	139.06 ± 43.31	
D (10)	127.03 ± 38.11	

ALT, alanine aminotransferase; AST, aspartate transaminase; BCLC, Barcelona Clinic Liver Cancer. [a]Sum may not always add up to total because of missing data.

positive patients with CHB (130.90 ± 49.49 μg/ml) than HBeAg-negative patients (111.39 ± 78.41 μg/ml) ($P < 0.05$) (**Figure 3A**).

Correlations between β2GPI and TH1/TH2 type cytokines in HBV infection patients with HCC, CHB and AHB

The potential associations between β2GPI and TH1/TH2 type cytokines were also analyzed in this study. As a result, no significantly correlations with β2GPI were found on the content of IFN-γ, TNF-α, IL-2, IL-4, IL-17A, IL-10 and IL-6 in patients with HCC and CHB (data was not shown). However, the plasma level of β2GPI was found to be significantly increased with IFN-γ in AHB patients, which exhibited an obviously positive correlation ($R=0.742$, $P=0.014$) (**Figure 3B**).

Discussion

β2GPI was known as a multifunctional apolipoprotein involved in HBV infection by interacting with HbsAg [17]. Meanwhile, it also played an important role in the development of HBV-related HCC [18, 19]. However, the clinical relevance of β2GPI on HBV-related liver diseases, especially HBV-related HCC, was still limited. In this study, plasma β2GPI was significantly increased in patients with HBV-related HCC, which exhibited a higher level than those in CHB and AHB patients. This finding was consisted with previous studies that β2GPI protein was up-regulated in HBV-related HCC, and further indicated a diagnosis potential on HBV-related HCC [20]. It has been reported high expression of β2GPI contributed to HBV infection by increased binding activity of HBsAg on cell surfaces [21]. β2GPI was regulated in a cell cycle-dependent manner with high level in proliferating cells, and considered to be a survival factor in hepatocytes by maintaining cell vitality in response to cellular stress [22]. Meanwhile, oxidative stress could also enhance the expression of β2GPI in hepatoma cells by AP-1 and NF-κB [23]. All these phenomena illustrated overexpressed β2GPI was interrelated with HBV-related HCC, and this correlation was closely related with HBV infection, elevated reactive oxygen species and rapid proliferation of cancer cells. Furthermore, the diagnosis performance of β2GPI was also demonstrated in this study, and the content of β2GPI was not influenced by AFP. What is important, an effective diagnosis role on HBV-related HCC was exhibited by combination of AFP and β2GPI. These findings suggest that β2GPI was a supplement to AFP for the differentiation of HBV-related HCC from other HBV-related liver diseases, and further support its clinical use in diagnosis and targeted therapy. However, the biological significance of β2GPI in pathogenesis of HBV-related HCC and detailed mechanisms remains to be studied.

The plasma level of β2GPI was always different in individuals, which ranged from 150 μg/ml to

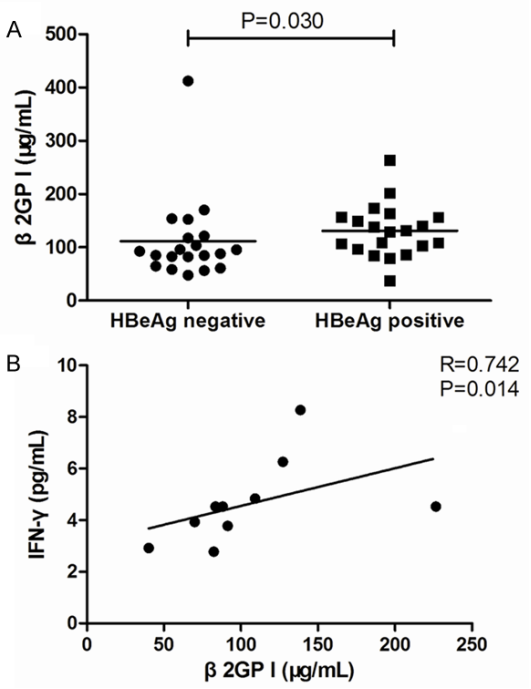

Figure 3. A. The plasma level of β2GPI in HBeAg-positive and -negative patients with chronic hepatitis B (CHB). B. A positive correlation between plasma level of β2GPI and IFN-γ in acute hepatitis B (AHB) patients.

200 μg/ml in black and white people [24]. In this study, a significantly lower β2GPI was exhibited in healthy northern Chinese (92.22 ± 12.65 μg/ml), which may be explained by the differences on detection methods, district and races of enrolled samples. On the other hand, plasma β2GPI was reported to be correlated with Child-Pugh stage and exhibited low level in patients with liver cirrhosis [25, 26]. However, no significant correlations with β2GPI were revealed on Child-Pugh stage and liver cirrhosis in this study. As our research was only performed on patients with HBV, the special relations with Child classification and liver cirrhosis may be neutralized by HBV induced high expression of β2GPI.

β2GPI was known to be closely interacted with HbsAg in CHB patients [21]. In this study, an interesting association was revealed between β2GPI and HBeAg, which has not been reported previously. The significantly higher level of β2GPI in HBeAg-positive CHB patients indicated a host factor role of β2GPI on HBV infection. However, special interaction mechanisms are still unclear, and further investigations on how HbeAg interacted with β2GPI were needed. On the other hand, it has been reported the serum profile of cytokines in hepatitis C virus (HCV) carriers presenting autoimmune markers may be mainly represented by increased IL-2, IL-5 and B-cell activating factor (BAFF) [27]. For HBV carriers, only a positive correlation between β2GPI and IFN-γ was revealed in AHB patients in our present research. This relation may be explained by a similar mechanism with anti-phospholipid syndrome that β2GPI stimulation could induce Th1 cells proliferation thereby secreting IFN-γ in peripheral blood mononuclear cells [28, 29].

Conclusion

β2GPI was significantly elevated in HBV infection patients with HCC, which could be used as a novel plasma diagnosis indicator in clinic. In addition, β2GPI was also associated with HbeAg in CHB patients and with IFN-γ in AHB patients. However, this study was still limited by insufficient population, and no correlations with β2GPI were revealed on clinical characteristics of HBV-related HCC. Further researches on the relations between β2GPI and HBV-related liver diseases, especially HBV-related HCC, and the related mechanisms were still needed in a large population.

Disclosure of conflict of interest

None.

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