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The Prognostic Significance of Plasma Beta2-Glycoprotein I Levels in Hepatocellular Carcinoma Patients

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Abstract. *Background/Aim: Beta2-glycoprotein I (β2-GPI) is a plasma glycoprotein with multiple physiological functions, but its relationship with hepatocellular carcinoma (HCC) is still poorly understood. HCC is one of the most common forms of liver cancer and is a leading cause of cancer-related death worldwide. This study aimed to investigate the association between β2-GPI and liver cancer and further validate its potential as a biomarker for HCC. Patients and Methods: Thirty-six patients diagnosed with HCC at the Division of Gastroenterology and Hepatology, E-Da Hospital, Taiwan, were included in the study. The expression levels of β2-GPI in plasma specimens from patients with HCC were determined by enzyme immunoassay and analyzed in relation to clinicopathological variables using the Chi-square test or Fisher's exact test. The predictive significance of β2-GPI for both overall survival (OS) and disease-free survival (DFS) was assessed using Kaplan-Meier estimates, and the statistical significance of differences was evaluated through the log-rank test. Cox proportional hazards regression models were used to evaluate the association between OS/DFS time and clinicopathological characteristics. Results: Plasma β2-GPI levels were significantly lower in patients with HCC compared to non-cancer controls and significantly correlated with aspartate aminotransferase (AST) levels of HCC. High plasma β2-GPI levels were significantly associated with better OS and DFS in HCC patients. Furthermore, in multiple variates analyses, OS was found to be significantly better in HCC patients with higher plasma β2-GPI expression. Conclusion: Elevated levels of β2-GPI protein in the plasma of HCC patients were identified as an independent factor predictive of improved OS and DFS. Activating β2-GPI in individuals at high risk could serve as a promising way for mitigating the progression of HCC.*

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Beta2-glycoprotein I (β2-GPI), also referred to as apolipoprotein H (apoH), is a human plasma glycoprotein with a molecular weight of approximately 50 kDa, consisting of 326 amino acids (1, 2). β2-GPI is primarily synthesized in the liver, and in plasma, about 35% of β2-GPI associates with lipids to form chylomicrons, very low-density lipoprotein (VLDL), and high-density lipoprotein (HDL) (3). Approximately 65% of β2-GPI remains in free form. β2-GPI exhibits diverse effects in conditions, such as antiphospholipid syndrome, autoimmune disorders, and oxidative stress (4-7). However, the exact role of β2-GPI has not yet been elucidated.

In previous studies, we discovered that β2-GPI suppresses melanoma cell migration, proliferation, and invasion *in vitro*, as well as inhibits melanoma growth *in vivo*. Additionally, we identified specific amino acid residues of β2-GPI that are involved in reducing the malignancy of melanoma cells (8). Furthermore, our preliminary results demonstrated that an inverse relationship between the expression of β2-GPI in breast cancer tissues and the risk of developing breast cancer in patients (9). Nevertheless, previous studies have suggested that β2-GPI may play a crucial role in tumor growth inhibition, indicating its potential significance in the development of cancer.

Liver cancer is a major malignancy with high mortality worldwide. Hepatocellular carcinoma (HCC), the predominant histological subtype, accounts for approximately 90% of all primary liver cancers (10, 11). Despite considerable efforts in the development of molecular-targeted therapies for HCC, prognosis remains unsatisfactory, largely due to late-stage diagnosis and intrahepatic metastasis (12). Surgical resection is known to improve overall survival (OS) in HCC; however, a significant portion of patients are ineligible for surgery primarily due to advanced metastasis (12). Therefore, understanding the molecular mechanisms underlying HCC pathogenesis and identifying potential diagnostic and prognostic biomarkers are crucial endeavors. In this study, we investigated the expression levels of β2-GPI in the plasma of HCC patients using an enzyme immunoassay. We explored the association between plasma β2-GPI levels and clinical variables to determine if β2-GPI levels could serve as a promising approach to determine progression of HCC.

Patients and Methods

Patients and samples. From November 2011 to March 2017, 36 patients with pathologically confirmed HCC were included in this study. HCC patients received surgery at the Division of Gastroenterology and Hepatology, E-Da Hospital, Taiwan. Histological classification was carried out using the modified classification system by the World Health Organization (WHO), while primary tumor grading was assessed using the Modified Bloom-Richardson Grading Scheme. Staging analysis was performed according to the AJCC TNM system. Disease-free survival (DFS) was calculated from the date of surgery to the date of local recurrence of liver cancer. OS was defined as the time from the date of surgery to the date of cancer-related death. This study was approved by the Institutional Review Board of E-Da Hospital (EMRP-113-001) and all patients had been informed and consented to the procedure before the operation.

Enzyme immunoassay. Plasma β2-GPI levels were assessed in duplicate for 36 HCC patients and 20 non-cancer control participants using a human β2-GPI-specific enzyme immunoassay kit (ab274403; Abcam, Cambridge, UK), following the manufacturer's instructions.

Statistical analysis. The difference of quantification of plasma β2-GPI expression between 36 HCC patients and 20 non-cancer control participants was evaluated by the Wilcoxon rank sum test. The patients' demographic and clinical characteristics among the β2-GPI groups were expressed as number and percentage. The cut-off value of plasma β2-GPI level was set to 143.98 pg/ml based on the 5% percentile of control subjects. In this study, patients with plasma β2-GPI levels less than 143.98 pg/ml were defined as the low-expression group, and values greater than or equal to 143.98 pg/ml was defined as the high-expression group. The high-expression and low-expression groups of β2-GPI were compared in terms of age, body mass index (BMI), tumor stage, tumor number, tumor size, hepatitis B surface antigen (HBsAg) status, aspartate aminotransferase (AST), alanine aminotransferase (ALT), AST/ALT ratio, and alpha-fetoprotein (AFP) using either the chi-square test or Fisher's exact test. Kaplan-Meier estimates were utilized to generate OS and DFS curves, and differences in survival curves between groups were assessed using the log-rank test. Hazard ratios (HR) and 95% confidence intervals (CI) were calculated using both univariate and multiple variates Cox proportional hazards regression models to evaluate the associations between survival time and clinicopathological characteristics. The statistical significance of all tests was evaluated at a predetermined significance level of 0.05. All data analyses were carried out using the SAS statistical software version 9.4 (SAS Institute Inc., Cary, NC, USA).

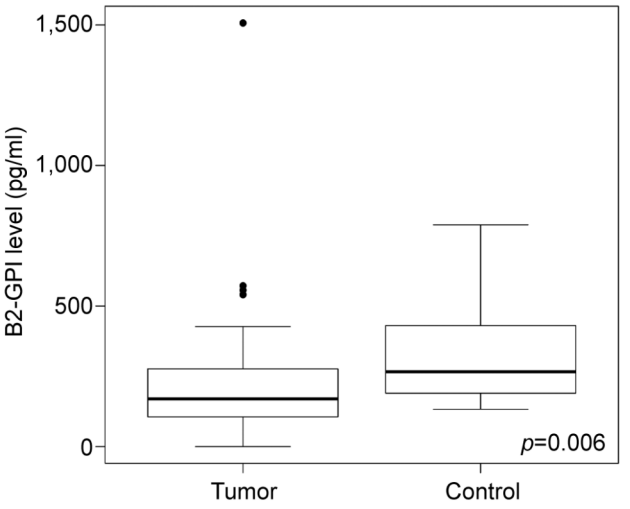

Figure 1. *Quantification of plasma β2-GPI expression using enzyme immunoassay in 36 hepatocellular carcinoma patients and 20 non-cancer control participants. Statistical significance was evaluated by the Wilcoxon rank sum test.*

Results

Plasma expression levels of β2-GPI in HCC patients. To investigate the plasma expression of β2-GPI protein in HCC patients, we conducted enzyme immunoassay on 36 HCC specimens and 20 non-cancer control participants. Our results showed that plasma β2-GPI was significantly down-regulated in HCC specimens compared to non-cancer control participants (Figure 1). To further investigate the plasma expression of β2-GPI protein in HCC, we analyzed its plasma expression in HCC from 36 patients by enzyme immunoassay, and correlated plasma β2-GPI expression levels with the clinicopathological characteristics of these patients. We categorized plasma β2-GPI protein expression levels in HCC into "low-" expression and "high-" expression groups based on a cut-off value of 143.98 pg/ml for the plasma β2-GPI level. Patients with plasma β2-GPI levels <143.98 were classified as having low β2-GPI expression, while patients with plasma β2-GPI levels ≥143.98 were categorized as having high β2-GPI expression.

Table I. *Associations of plasma B2-GPI levels with demographic and clinical characteristics in HCC patients.*

			B2-GPI				
			Low		High		
Variables	Item	Patient No. (%)	N	%	N	%	*p*-Value*
		36 (100)	13	36.1	23	63.9	
Age (years)	<65	18 (50.0)	6	46.2	12	52.2	0.729
	≥65	18 (50.0)	7	53.8	11	47.8	
BMI (kg/m^2)	<24	14 (38.9)	6	46.2	8	34.8	0.501
	≥24	22 (61.1)	7	53.8	15	65.2	
T stage	T1	22 (61.1)	8	61.5	14	60.9	0.969
	T2, T3 or T4	14 (38.9)	5	38.5	9	39.1	
N stage	N0	34 (94.4)	12	92.3	22	95.6	1.000
	N1	2 (5.6)	1	7.7	1	4.4	
M stage	M0	32 (88.9)	11	84.6	21	91.3	0.609
	M1 or Mx	4 (11.1)	2	15.4	2	8.7	
HBsAg	Negative	26 (78.8)	10	83.3	16	76.2	1.000
	Positive	7 (21.2)	2	16.7	5	23.8	
Tumor number	Single	24 (66.7)	9	69.2	15	65.2	1.000
	Multiple	12 (33.3)	4	30.8	8	34.8	
Tumor size (cm)	<2.95	18 (50.0)	5	38.5	13	56.5	0.298
	≥2.95	18 (50.0)	8	61.5	10	43.5	
AST (U/l)	≤40	9 (25.0)	0	0.0	9	39.1	0.014
	>40	27 (75.0)	13	100.0	14	60.9	
ALT (U/l)	≤40	17 (47.2)	4	30.8	13	56.5	0.137
	>40	19 (52.8)	9	69.2	10	43.5	
AST/ALT ratio	≤1.24	19 (52.8)	6	46.2	13	56.5	0.550
	>1.24	17 (47.2)	7	53.8	10	43.5	
AFP (ng/ml)	≤200	19 (54.3)	7	53.8	12	54.5	0.968
	>200	16 (45.7)	6	46.2	10	45.5	

**p*-Value calculated by the Chi-squared test or Fisher's exact test. HCC: Hepatocellular carcinoma; B2-GPI: beta2-glycoprotein I; BMI: body mass index; HBsAg: hepatitis B surface antigen; AST: aspartate aminotransferase; ALT: alanine aminotransferase; AFP: alpha-fetoprotein.

Association between the plasma expression pattern of β2-GPI and clinicopathological characteristics of HCC patients. The associations between the expression levels of β2-GPI and the clinicopathological characteristics in HCC patients are shown in Table I. We found that high β2-GPI expression levels in HCC patients were significantly associated with low AST values (p=0.014) (Table I). Age, BMI, tumor stage, HBsAg status, tumor number, tumor size, ALT, AST/ALT ratio, and AFP were not found to be significantly correlated with plasma β2-GPI expression.

Relationship between β2-GPI expression in plasma and survival in HCC patients. Survival analysis by the log-rank test demonstrated increased OS and DFS rates in the high plasma β2-GPI expression group compared with the low plasma β2-GPI expression group (p=0.008 and p=0.038, respectively) (Figure 2). We also evaluated the risk factors associated with HCC. To investigate the relationship between plasma β2-GPI expression and clinicopathological parameters with OS, both univariate and multiple variates Cox proportional hazards regression models were performed (Table II). In the univariate analysis, AST/ALT ratio (>1.24 *vs.* ≤1.24, HR of death=3.27, p=0.048), AFP (>200 ng/ml *vs.* ≤200 ng/ml, HR of death=3.35, p=0.048), and plasma β2-GPI expression (high *vs.* low expression, HR of death=0.2, p=0.017) showed statistically significant associations with OS. In the multiple variates analyses, statistically significant relationships with OS were observed for one parameter: β2-GPI expression (high *vs.* low expression, HR of death=0.25, p=0.047). Similar results were observed for DFS by univariate and multiple variates analyses (Table III). In the univariate analysis, AST (>40 U/l *vs.* ≤40 U/l, HR of death=3.53, p=0.049), ALT (>40 U/l *vs.* ≤40 U/l, HR of death=2.74, p=0.041), and plasma β2-GPI expression (high *vs.* low expression, HR of death=0.39, p=0.045) were statistically significantly associated with DFS. In multiple variates analyses, there were no significant independent risk factors for DFS.

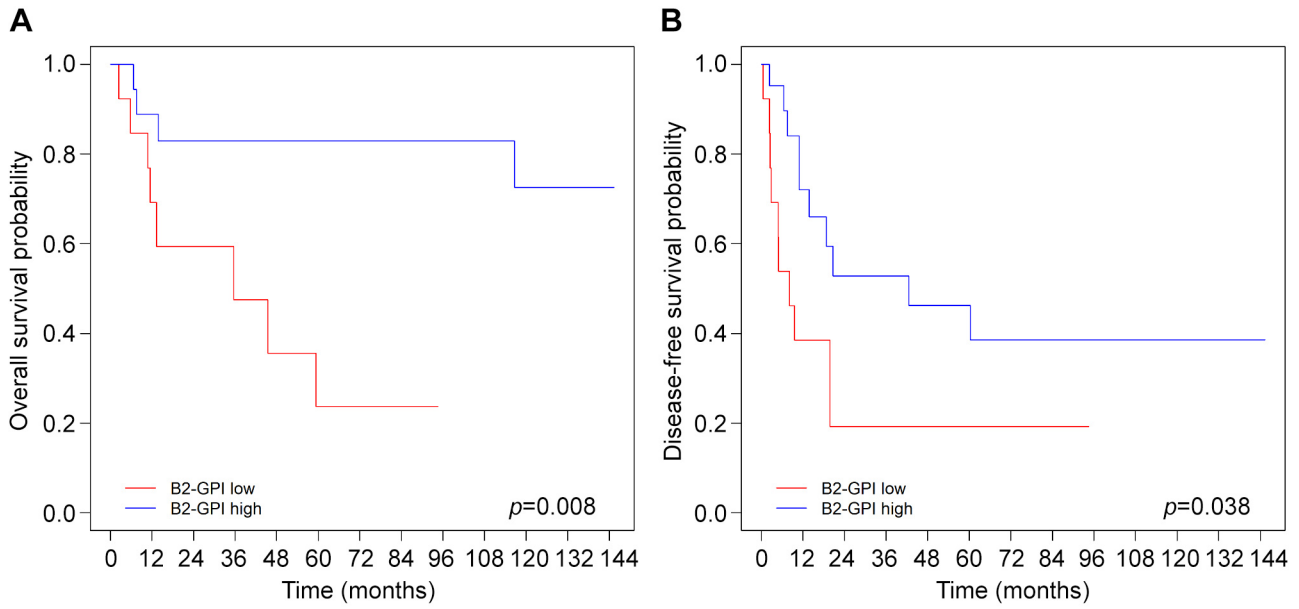

Figure 2. *Overall survival (A) and disease-free survival (B) for HCC patients with high versus low plasma β2-GPI expression. Survival curves were estimated by the Kaplan–Meier method and the p-values were evaluated using a log-rank test.*

Table II. *Univariate and multiple variates analysis of overall survival for HCC patients.*

Variables	Item	Univariate			Multiple variates*		
		HR	95% CI	*p*-Value	HR	95% CI	*p*-Value
Age (years)	≥65	1.27	(0.39, 4.08)	0.694	-	-	-
	<65	1.00			-		
BMI (kg/m^2)	≥24	1.07	(0.32, 3.57)	0.918	-	-	-
	<24	1.00			-		
T stage	T2, T3 or T4	1.44	(0.43, 4.83)	0.557	-	-	-
	T1	1.00			-		
M stage	M1 or Mx	2.46	(0.29, 21.06)	0.412	-	-	-
	M0	1.00			-		
HBsAg	Positive	0.39	(0.05, 3.03)	0.365	-	-	-
	Negative	1.00			-		
Tumor number	Multiple	1.60	(0.48, 5.33)	0.447	-	-	-
	Single	1.00			-		
Tumor size (cm)	≥2.95	1.29	(0.41, 4.02)	0.661	-	-	-
	<2.95	1.00			-		
AST (U/l)	>40	6.06	(0.78, 47.38)	0.086	-	-	-
	≤40	1.00			-		
ALT (U/l)	>40	2.35	(0.70, 7.90)	0.169	-	-	-
	≤40	1.00			-		
AST/ALT ratio	>1.24	3.27	(1.01, 10.55)	0.048	2.86	(0.84, 9.72)	0.093
	≤1.24	1.00			1.00		
AFP (ng/ml)	>200	3.35	(1.01, 11.10)	0.048	3.72	(0.99, 13.98)	0.052
	≤200	1.00			1.00		
B2-GPI	High	0.20	(0.05, 0.74)	0.017	0.25	(0.07, 0.98)	0.047
	Low	1.00			1.00		

*Variables with p≤0.05 on univariate analysis were included in multiple variates analysis. HCC: Hepatocellular carcinoma; HR: hazard ratio; CI: confidence interval; BMI: body mass index; HBsAg: hepatitis B surface antigen; AST: aspartate aminotransferase; ALT: alanine aminotransferase; AFP: alpha-fetoprotein; B2-GPI: beta2-glycoprotein I.

Table III. *Univariate and multiple variates analysis of disease-free survival for HCC patients.*

Variables	Item	Univariate			Multiple variates*		
		HR	95% CI	*p*-Value	HR	95% CI	*p*-Value
Age (years)	≥65	1.62	(0.65, 4.04)	0.302	-	-	-
	<65	1.00			-		
BMI (kg/m^2)	≥24	1.07	(0.42, 2.73)	0.884	-	-	-
	<24	1.00			-		
T stage	T2, T3 or T4	1.74	(0.64, 4.76)	0.279	-	-	-
	T1	1.00			-		
M stage	M1 or Mx	1.45	(0.19, 11.23)	0.721	-	-	-
	M0	1.00			-		
HBsAg	Positive	0.51	(0.12, 2.27)	0.380	-	-	-
	Negative	1.00			-		
Tumor number	Multiple	2.19	(0.81, 5.92)	0.124	-	-	-
	Single	1.00			-		
Tumor size (cm)	≥2.95	1.07	(0.43, 2.69)	0.879	-	-	-
	<2.95	1.00			-		
AST (U/l)	>40	3.53	(1.01, 12.37)	0.049	1.48	(0.27, 8.09)	0.653
	≤40	1.00			1.00		
ALT (U/l)	>40	2.74	(1.04, 7.20)	0.041	2.21	(0.65, 7.57)	0.205
	≤40	1.00			1.00		
AST/ALT ratio	>1.24	1.58	(0.64, 3.94)	0.323	-	-	-
	≤1.24	1.00			-		
AFP (ng/ml)	>200	2.45	(0.94, 6.36)	0.067	-	-	-
	≤200	1.00			-		
B2-GPI	High	0.39	(0.15, 0.98)	0.045	0.46	(0.16, 1.33)	0.150
	Low	1.00			1.00		

*Variables with p≤0.05 on univariate analysis were included in multiple variates analysis. HCC: Hepatocellular carcinoma; HR: hazard ratio; CI: confidence interval; BMI: body mass index; HBsAg: hepatitis B surface antigen; AST: aspartate aminotransferase; ALT: alanine aminotransferase; AFP: alpha-fetoprotein; B2-GPI: beta2-glycoprotein I.

Discussion

Hepatectomy is the primary treatment for HCC worldwide. However, due to limitations in diagnostic techniques, early detection of HCC is often challenging, leading to poor prognosis. Currently, the five-year postoperative recurrence rate remains high (13). To improve prognosis of HCC, many researchers are conducting various studies. Studies have indicated that elevated levels of certain markers may be associated with the prognosis of HCC patients. A recent report has shown that elevated Sjögren's syndrome nuclear autoantigen-1 (SSNA1) expression in HCC patients was closely associated with a poor prognosis (14). Likewise, high kinesin family member 15 (KIF15) expression in inflammatory monocytes within tumor tissues may serve as a prognostic marker for poor outcomes in HCC (15). However, Koh *et al*. found that low transient receptor potential vanilloid 6 (TRPV6) expression predicted an adverse prognosis following curative HCC resection (16). Furthermore, prognostic factors, such as TNM stage, tumor size, vascular invasion, and recurrence rate have been identified (17-19). However, these factors are difficult to assess before surgery, prompting extensive research into prognostic plasma markers in recent years.

Recent studies have shown that mice lacking β2-GPI expression exhibit increased microvessel formation and accelerated melanoma tumor growth (20). We further investigated the protective role of β2-GPI in regulating B16-F10 melanoma cells through *in vitro* and *in vivo* experiments (8). In our recent findings, a relationship between β2-GPI expression in tissues and prognosis has been shown in breast cancer (9). However, there has been no research on plasma β2-GPI expression in HCC and its relationship with patient survival. In this study, we examined the role of β2-GPI in HCC by analyzing its expression patterns in the plasma and correlating these patterns with clinical characteristics, OS, and DFS in HCC patients.

In our current study, we found that plasma β2-GPI levels were significantly lower in HCC patients compared to non-cancer control participants (Figure 1). Analysis of clinicopathological parameters revealed that reduced β2-GPI levels were associated with AST status (Table I). Specifically,

lower β2-GPI levels were consistently linked to higher AST. AST and ALT are important liver enzymes (21). ALT is primarily located in the non-mitochondrial portion of hepatocytes, while AST is predominantly found in the mitochondria of hepatocytes. In advanced liver disease, mitochondrial damage can occur, releasing AST into the bloodstream and significantly increasing its plasma levels. Additionally, as liver function deteriorates, the clearance rate of AST decreases, further elevating plasma AST levels compared to ALT levels (22-24). Our data showed that significantly increased plasma expression levels of AST (>40 U/l) were observed in HCC patients (Table I), especially among patients with low plasma β2-GPI expression. In our research, HCC patients with higher AST/ALT ratios had poorer prognoses compared to those with lower ratios (Table II), identifying AST/ALT as a risk factor for OS in HCC patients. AST/ALT ratio is closely linked to residual hepatic inflammatory necrosis (25-27). In the multiple variates analyses (adjusted for AST/ALT ratio and AFP), the HR of OS in the high plasma β2-GPI expression group was 0.25 and that of OS in the low plasma β2-GPI expression group was 1.0 (Table II), indicating that plasma β2-GPI expression levels predominantly affects HCC patients' OS. These findings suggest that β2-GPI may play a beneficial role in HCC. Our results align with previous studies indicating that high β2-GPI expression acts as a negative regulator in breast cancer patients (9). In HCC, high β2-GPI expression may offer protective effects for patients. However, the specific mechanisms by which plasma β2-GPI levels influence the prognosis of HCC patients require further investigation.

Plasma AFP levels are frequently elevated in patients with HCC, making AFP a commonly used surrogate marker for the disease (28, 29). Elevated AFP levels are associated with a higher risk of developing HCC. In our present investigation, analysis of clinicopathological parameters revealed that plasma β2-GPI levels were not correlated with AFP levels (Table I). Notably, we found that in the univariate analysis, the HR of OS in the AFP levels >200 ng/ml group was 3.35 and that in the AFP levels ≤200 ng/ml group was 1.0 (Table II). The significance of β2-GPI expression was further underscored by multiple variates Cox regression analyses, which showed that after adjusting for AST/ALT ratio, AFP levels, and β2-GPI expression, only β2-GPI expression remained statistically significant in relation to OS in HCC patients (Table II). Moreover, our clinicopathological analyses demonstrated a negative correlation between plasma β2-GPI levels and HBsAg status in HCC patients (Table I). Jing *et al*. suggested that β2-GPI may contribute to development of HBV-related HCC by activating NF-κB through its interaction with HBsAg (30), and a later study demonstrated that HBsAg/β2-GPI activates the NF-κB pathway through the TLR4/MyD88/IκBα axis in HCC (31). Interestingly, our previous study showed that oxidative stress amplifies the regulation of β2-glycoprotein I gene expression in hepatoma cells through AP-1 and NF-κB pathways (32). Further research and patient stratification based on HBsAg status could offer deeper insights into risk assessment and survival outcomes for patients with different levels of β2-GPI. The primary limitation of our study was its small sample size, which affects the generalizability of our results. To address this, we performed a post-hoc power analysis to evaluate the statistical power of log-rank test results at a significance level of 0.05. The achieved power for overall survival and disease-free survival was 84% and 99%, respectively, with a sample size of 36 HCC patients. These analyses confirm that our methods have adequate power for detecting significant results. Additionally, the retrospective and single-center design must be considered. Furthermore, our cohort only spans a period of nearly 7 years.

To the best of our knowledge, this is the first study to propose the predictive value of β2-GPI for OS and DFS in HCC patients. Our findings revealed that the adjusted hazard ratios for OS and DFS in HCC patients with high β2-GPI expression were 0.25 and 0.46, respectively (Table II and Table III). Additionally, high β2-GPI expression in the plasma was linked to improved OS and DFS rates (Figure 2). Identifying protective factors like β2-GPI, as demonstrated in our study, may provide valuable insights for physicians in developing more effective HCC prevention strategies.

Conclusion

Our study indicates a negative association between plasma β2-GPI expression and HCC risk. Elevated plasma β2-GPI expression was found to be an independent predictor of improved survival outcomes, including OS and DFS, in HCC patients. These findings suggest that β2-GPI may play a protective role in lowering the risk of HCC.

Conflicts of Interest

The Authors declare that they have no conflicting interests.

Authors' Contributions

TJH and WCC conceived and designed the study; TJH, GYH, and WCC analyzed the data; YJH and WCC drafted the manuscript; TJH, YNT, YYL, HWP, YCH, and WCC provided material support and study supervision. All Authors reviewed and approved the final manuscript.

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