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ORIGINAL ARTICLE

Serum sphingosine 1-phosphate in hepatocellular carcinoma patients is related to HBV infection

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Summary

Purpose: *Serum predictors for early diagnosis of hepatocellular carcinoma (HCC) have been investigated. Sphingosine-1-phosphate (S1P) has been widely reported to promote the survival of many types of cancer cells. However, the potential of serum S1P as a diagnostic marker in HCC has not been well characterized. The aim of this study was to identify the relationship between serum S1P and the risk of HCC.*

Methods: *We retrospectively reviewed serum S1P in 63 HCC patients and 39 normal people. Receiver operating characteristic (ROC) curve analysis was performed to define the cut-off value of S1P in the serum. Chi-square test, t-test and multivariate regression analysis were used to investigate the association between serum S1P and individual clinicopathologic parameters.*

Results: *S1P showed significantly higher level in healthy subjects (1.372±0.116 μM) than that in patients (1.372±0.116 μM). Serum S1P in HCC patients was positively correlated to globulin (t=-3.122, p=0.003), hepatitis B virus (HBV) DNA copies (x^2=4.386, p=0.036) and negatively related to AST (x^2=2.870, p=0.09). Besides, part of the amount of serum S1P was negatively correlated to albumin (correlation coefficient (β)=-0.056) and positively correlated to alanine aminotransferase (ALT) (β=0.016) according to the regression analysis.*

Conclusions: *These results suggested that serum S1P could be used as an auxiliary marker for HCC diagnosis, and used to monitor HBV infection in patients with HCC.*

Key words: *HBV, hepatocellular carcinoma, liver damage, serum sphingosine 1-phosphate*

Introduction

According to recent reports (2012), hepatocellular carcinoma (HCC) constitutes a major health problem since it represents the sixth most common cancer and the third leading cause of cancer related mortality worldwide [1,2]. Despite the progress made in diagnostic techniques and surgical instruments, the prognosis remains far from satisfactory because of the high rate of recurrence and metastasis [3]. Accurate early diagnosis is the key to improve the prognosis of HCC. However, the level of α-fetoprotein (AFP) in serum which has been used as predictor of liver cancer for a long time is not so qualified for diagnosis.

It was known that S1P worked as an extracellular mediator to activate G protein coupled receptors. Accordingly, it acts on at least 5 receptors (S1PR1–5) with high affinity [4]. As a circulating paracrine mediator, S1P, which is stored and re-

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leased from platelets or erythrocytes, has normal internal functions as well as potentially pathophysiological roles when it recognized the receptor [5,6]. Recently, increasing evidence indicated that S1P correlated with the pathophysiology of cancer and played an essential role in cancer development [7]. Especially S1P is required for vascular development and tumor angiogenesis [8]. Meanwhile intracellular S1P is an important cell death regulator [9] as it has an inhibitory effect on hepatocyte proliferation [10,11]. Besides, serum S1P was associated with hepatic fibrosis [6], in which it enhances portal vein pressure [12]. However, the potential for the serum S1P as a HCC marker is not well investigated.

Serologically, approximately 15% of all patients with HCC are reported to be hepatitis B surface antigen (HBsAg)-positive [13]. HBV itself has direct carcinogenic potential, and studies have shown that HBV might directly activate oncogenic signaling [14]. Therefore, the activity of HBV is present as a useful indicator showing the risk of HCC development.

In this research, serum S1P in 63 HCC patients and 39 normal people was reviewed retrospectively. The results showed that serum S1P in healthy donors was significantly higher than that in HCC patients. Meanwhile, serum S1P was related to the number of HBV DNA copies and the level of globulin in HCC patients. Besides, the linear relationship among serum S1P, albumin and ALT was revealed in this manuscript. It was suggested here that serum S1P could be used as an auxiliary marker for HCC diagnosis, and used to monitor HBV infection in patients with HCC.

Methods

Study population

HCC samples with complete clinical and pathological data were collected from 63 HCC patients at the Affiliated Hospital of Guilin Medical University, Guangxi, China, between February 2014 and April 2016. All of the preoperative patients had conventional assessments including complete hematologic and serum biochemistry tests, physical examination, ultrasonography (US), computed tomography (CT) scans, and magnetic resonance imaging (MRI) scans. Patient baseline and clinical data, including age, gender, HBsAg, AFP, median size of tumor, cirrhosis, tumor lesions number, clinical TNM stage, portal vein tumor thrombus (PVTT), and distant metastasis is listed in Table 1. The survey protocols complied with the ethical guidelines of the Declaration of Helsinki. Ethical approval was granted by the Ethical Committee of the Affiliated Hospital of Guilin Medical University, and written informed consent was obtained from all examined patients or their guardians prior to surgery.

Table 1. The relationship between S1P and clinicopathologic variables of patients with HCC

		No. of patients	*Serum S1P (μM)*		x^2	*p value*
			≤1.8493	*>1.8493*		
HBV DNA copies	≤1000	29	26 (89.7)	3 (10.3)	4.386	0.036
	>1000	34	23 (67.6)	11 (32.4)		
Size (cm)	≤6	26	19 (73.1)	7 (26.9)	0.566	0.452
	>6	37	31 (81.1)	7 (18.9)		
AFP (μg/l)	≤20	24	18 (75.0)	6 (25.0)	0.173	0.677
	>20	39	31 (79.5)	8 (20.5)		
AST (U/L)	≤40	35	30 (85.7)	5 (14.3)	2.870	0.09
	>40	28	19 (67.9)	9 (32.1)		
LYM ratio (%)	≤1.3	62	48 (77.4)	14 (22.6)	1000	
	>1.3	1	1 (100)	0 (0)		
Albumin (g/L)	≤35	20	15 (75.0)	5 (22.2)	0.131	0.718
	>35	43	34 (79.1)	9 (20.9)		
Globulin (g/L)	≤35	40	33 (82.5)	7 (17.5)	1.414	0.234
	>35	23	16 (69.6)	7 (30.4)		
Leukocyte	≤10×10^9/l	57	44 (77.2)	13 (22.8)	0.118	0.731
	>10×10^9/l	6	5 (83.3)	1 (16.7)		
A/G	≤1.3	39	30 (76.9)	9 (23.1)	0.043	0.835
	>1.3	24	19 (79.2)	5 (20.8)		

Selection of cutoff score

ROC curve analysis was used to determine the cut-off value of preoperative serum S1P in HCC patients. The optimal cutoff value was closest to the point with maximum sensitivity and specificity. To perform this analysis, we first dichotomized the rest of clinicopathological features, then further investigated the clinicopathologic significance of serum S1P in HCC.

Statistics

The statistical analyses were performed using SPSS19.0 (SPSS Inc, Chicago, IL). ROC curve analysis was applied to determine the cutoff value of preoperative S1P content by 0,1-criterion. Pearson x^2 test or continuity correction x^2 test were used to analyze the correlation between S1P content and clinicopathological parameters.

Results

Serum S1P decreased in HCC patients

S1P is the cancer cells' promoter but its fluctuations in the serum in HCC patients are not fully investigated. To figure out the exact relationship between serum S1P and the risk of HCC, S1P in HCC patients and normal people were measured. To our surprise, serum S1P in HCC patients was decreased to 1.372 ± 0.919 μM (n=63; 95% CI, 1.14–1.60), with the lowest level down to 0.14 μM, significantly lower than 3.196 ± 1.606 μM (n=39; 95% CI, 2.68–3.72) in healthy donors (t=-6.47, p< 0.001) (Figure 1A).

Identification of the cutoff value of preoperative serum S1P in healthy donors

To determine an optimal cut-off value of preoperative S1P in serum, a ROC curve was produced by SPSS software to validate the diagnostic value of this model which suggested that the score of 1.849 has the maximum sensitivity and specificity for indicating survival status. The area under receiver operating curves (AUC) was 0.831 with 95% CI of 0.739 to 0.924, a sensitivity 84.6%, and a specificity 76.2% (Figure 1B).

Stratified analysis according to globulin content

Patients were stratified according to globulin content for comparing the S1P content in two different subgroups. The data demonstrated that serum S1P was significantly lower in HCC patients with less globulin (normal) content compared to that in patients with more globulin content in the serum (0.726±0.115, 1.059±0.221, respectively) (t=-3.122, p=0.003, Figure 2). This result suggested that serum S1P represented the intensity of humoral immunity to some extent.

Association between S1P and clinicopathological features

The relationship between S1P and clinicopathologic variables of HCC patients was investigated after the generation of the cutoff value of serum S1P. The data showed that S1P was correlated with HBV copies (x^2=4.386, p=0.036) and

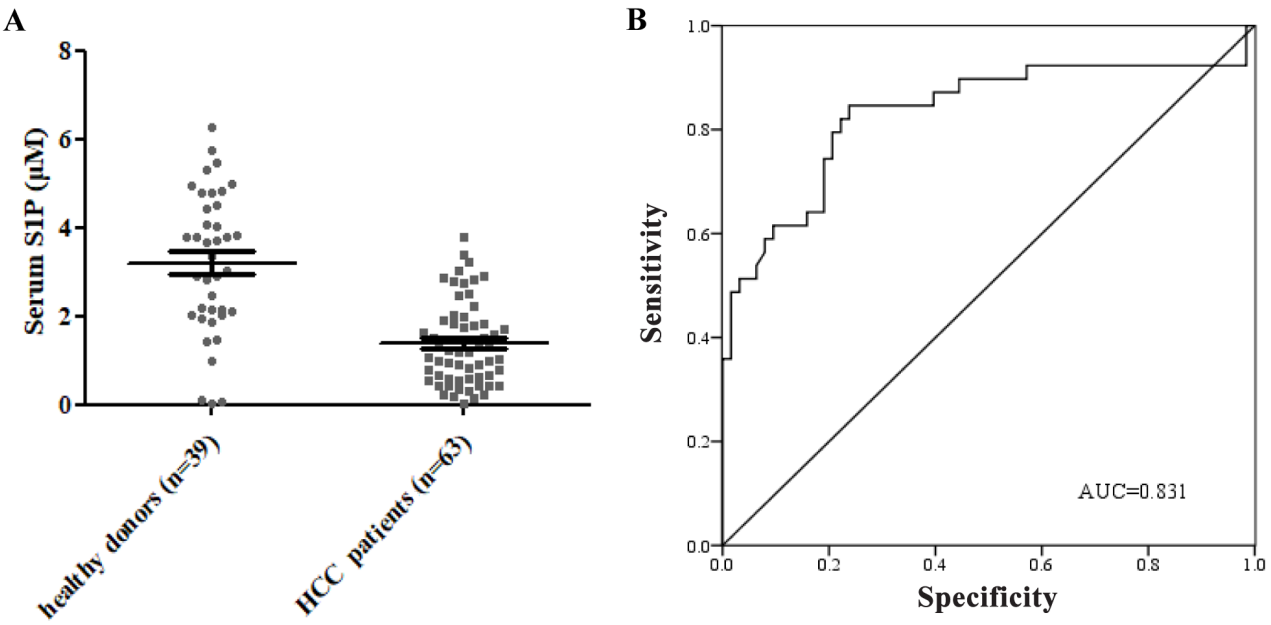

Figure 1. ROC curve and the distribution of S1P. **A:** The distribution of S1P in serum of HCC patients and normal individuals (p<0.001). **B:** ROC analysis was performed to evaluate S1P in the serum between hepatocellular carcinoma (HCC) patients and healthy persons. The area under the ROC curve value was 0.831.

AST (x^2=2.870, p=0.09). Nonetheless, there were no statistical connections between S1P and other clinicopathological parameters including age, gender, grade of differentiation, tumor nodules' number, capsular formation, TNM stage, BCLC stage, cirrhosis, PVTT, HBsAg, HBV DNA copies, size, AFP, aspartate aminotransferase, lymphocyte ratio, albumin, globulin, leukocytes, A/G (albumin to globulin ratio) (all p>0.05, Table 1). Taken together, it was indicated that serum S1P was also an indicator of HBV activity.

Linear relationship among S1P, alanine aminotransferase and albumin

To investigate the correlation of S1P with HBV infection as well as liver function in HCC patients, a linear regression method was applied. It was found that serum S1P in patients with HCC was positively correlated with albumin but negatively correlated with ALT content. Meanwhile other clinical characteristics of the fitting analysis were not relevant (Table 2).

Discussion

Our current study demonstrated that S1P might be a potential auxiliary predictor for the risk of HCC development. The ROC analysis suggested that 1,849 might be the most optimal reference value for S1P with a sensitivity of 84.6% and a specificity of 76.2% for healthy status indication. Of note, in HCC patients S1P was positively correlated to HBV DNA copies, globulin and ALT, while it was negatively correlated to albumin, all of which were linked to HBV infection.

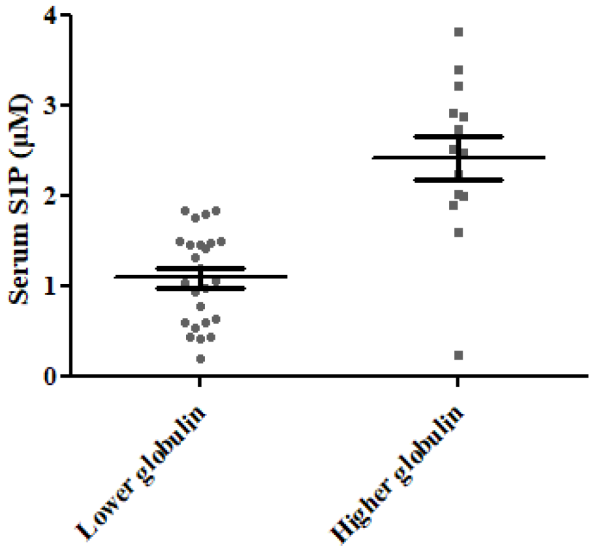

Figure 2. All 63 cases of HCC patients were stratified based on globulin content in serum (p<0.05).

It was confusing that as a tumor promotor S1P was found to decrease in the serum of HCC patients. This phenomenon can be easily understood since it was reported that S1P levels in HCC tissues are reduced. It might be the increased sphingosine kinase (SK) and S1P lyase (SPL) activity in HCC tissues resulted in downregulation in S1P from tissue and serum since this sphingolipid could be secreted from cancer cells [15].

Apart from erythrocytes, the main resource of serum S1P, endothelial cells, thrombocytes and various immunological cells can also release S1P [16]. The results showed that serum S1P did not correlate to erythrocytes or lymphocytes. So, here the fluctuation of serum S1P was probably due to the changes in sphingolipids metabolism in liver cancer cells.

AFP, CEA, and CA19.9 can be treated as tumor biomarkers, reflecting cancer cells' growth, differentiation, invasion and metastasis to some degree [17,18]. However, the diagnostic value of these markers has been questioned, for example, serum AFP did not significantly increase in 30% of HCC patients [19], multiple factors affected the diagnostic accuracy and reliability of CEA [17], and a high level of serum CA19.9 is frequently seen in healthy persons [18]. As our research showed that S1P is reduced in the serum of patients with HCC, decreased serum S1P could be used as an auxiliary indicator for HCC.

Another risk factor for HCC is HBV, which does not directly damage the hepatocytes but integrates its genome into the host chromosomal DNA and activates the immune system. The persistence of infection and liver damage induced by immunoreaction is accelerated [20]. Liver damage finally results in upregulation in AST and ALT secretion and downregulation in albumin secretion. In this study we revealed that serum S1P was positively correlated to HBV copies and ALT, but negatively cor-

Table 2. Linear regression results

Model	*F value*	*p value*	*Correlation coefficient (β)*	*t value*	*p value*	*Sum of squares*
(constant)			2.880	3.340	0.001	
ALT	6.912	0.020	0.016	3.154	0.003	0.187
Albumin			-0.056	-2.410	0.019	

related to albumin, therefore it could be concluded that serum S1P in HCC patients was a marker of the liver damage induced by HBV infection.

In case of HBV infection, both cellular and humoral immunity are activated to eliminate the virus. Globulin in serum represents the overall intensity of humoral immunity. Since S1P in serum from HCC patients was positively associated with globulin, therefore increased S1P could reflect the activation of humoral immunity. Of note, the large amount of $CD8^+$ T cells, especially intrahepatic HBV-specific $CD8^+$ T, play a critical role in HBV clearance [21]. Meanwhile, S1P was reported to promote the migration of immune cells to the lesion [22]. However, the total amount of lymphocytes was not related to serum S1P in HCC patients. Therefore, globulin increase might be due to the B cell migration or $CD8^+$ T cells activation induced by S1P.

It should be mentioned here that higher level of S1P indicated more HBV virus infection and stronger liver damage in HCC patients, while the level of serum S1P was even higher in normal subjects. The phenomenon provided supplementary evidence for the role of serum S1P as an immunity defense indicator in HCC patients with HBV infection.

In conclusion, serum S1P was significantly lower in HCC patients which could be used as a supplement for the HCC diagnosis. Besides, this sphingolipid was positively correlated to HBV DNA copies, ALT and globulin, but negatively correlated to albumin, based on which the role of serum S1P in predicting the immunity activation in HCC patients with HBV infection was identified.

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Conflict of interests

The authors declare no conflict of interests.

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