The 6th International Conference on Biochemistry and Molecular Biology

The diagnostic and prognostic roles of serum sulfatase-2 in patients with HBV-related hepatocellular carcinoma

Pimthong Thaweethongkam[1], Natthaya Chuaypen[1], Sunchai Payungporn[1], Pisit Tangkijvanich[1,*]

[1] Center of Excellence in Hepatitis and Liver Cancer, Department of Biochemistry, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand
*E-mail: pisittkvn@yahoo.com

Abstract

Hepatocellular carcinoma (HCC) is a primary liver cancer that mostly arises in patients with chronic hepatitis B virus (HBV) infection. Recently, upregulated expression of sulfatase-2 (SULF-2), an extracellular enzyme promoting tumor proliferation, has been reported in HCC tissues. The aims of this study were to evaluate the diagnostic and prognostic roles of serum SULF-2 in patients with HBV-related HCC. Three groups were studied, which included 146 patients with HCC, 119 patients with non-malignant chronic HBV infection and 50 healthy subjects. Serum SULF-2 and alpha-fetoprotein (AFP) levels were measured by enzyme-linked immunosorbent assay (ELISA) method. The HCC group was significantly older with higher proportion of male compared with the non-HCC group and healthy controls (P<0.001). Patients with HCC had higher levels of serum SULF-2 than those without HCC and controls (27.3 ± 10.3 vs. 18.5 ± 5.2 vs. 15.8 ± 4.3 ng/ml, P<0.001). The area under the curve (AUROC) for differentiating HCC from the other groups were 0.79 (95%CI; 0.73-0.84, P<0.001) for SULF-2 and 0.90 (95%CI; 0.86-0.94, P<0.001) for AFP. In the HCC group, serum SULF-2 levels positively correlated with AFP levels (r=0.286, P=0.001), Child-Pugh classification (r=0.206, P=0.016), tumor size (r=0.277, P=0.001) and tumor stage (BCLC stage) (r=0.274, P=0.001). High SULF-2 level (above median value as a cut-off point of 25 ng/ml) was significantly correlated with poor overall survival and was a prognostic factor in patients with HCC. Significant correlations of serum SULF-2 levels with tumor size and stage suggested that this marker might play important roles in promoting HCC progression. These findings also indicated that serum SULF-2 could serve as a diagnostic and prognostic marker for patients with HBV-related HCC.

Introduction

Hepatocellular carcinoma (HCC) is a primary liver cancer ranking the sixth globally [1] and the second cancer-related mortality among the other types of cancer[2]. In Thailand, the incidence of this primary liver cancer is 22.3 per 100,000/year and the mortality rate of patients is 21.5 per 100,000/year [3]. HCC usually occurs as a consequence of several risk factors including chronic hepatitis virus infection, excess alcohol assumption, metabolic or genetics aberration[4]. In Asia including Thailand, chronic hepatitis B virus (HBV) infection is the major risk factor for HCC [5, 6]. Chronic HBV infection causes injuries within the liver which leads to persistent inflammation, cirrhosis and finally HCC [7].

Generally, there are several types of treatments for HCC that are improved to enhance the efficacy and achievement of medication. The option of therapeutics for patients with the disease depends on the stage of HCC at the period of diagnosis [8]. According to the Barcelona

Clinic Liver Cancer (BCLC) staging and treatment guideline, the curative treatment potentially occurs in patients who were identified HCC at the early stage while palliative care was offered for patients with the end stage [9, 10]. Thus, diagnostic tools that able to identify HCC promptly are crucial for the survival of patients. Recently, the most common biomarker for HCC is alpha-fetoprotein (AFP) [11]. AFP is an oncofetal serum protein and usually detected less than 20 ng/ml in healthy person but significantly higher in HCC patients [11]. However, serum AFP might not be sensitive in detecting early stage HCC and its level may be also elevated in non-malignant chronic liver diseases, including chronic hepatitis and cirrhosis [12,13, 14]. Thus, identification of other serum markers HCC is necessary, particularly in countries with low-resource settings with high prevalence of HCC.

Sulfatase-2 (SULF-2) is an extracellular sulfatase enzyme that can modify the sulfate group at 6-O position of glucosamine residue of heparan sulfate proteoglycan (HSGAG) molecules [15, 16]. This position can be added with sulfate group besides the other positions including position N- and 3-O of glucosamine and the position 2-O of the iduronic acid [17]. The particular sulfation pattern of HSGAGs affects the binding capacity between them and their ligands that consequently alters the downstream activities. Thus, the modification of HSGAGs via SULF-2 can enhance the Wnt/β-catenin, Fibroblast growth factor (FGF) and extracellular signal regulated kinase (ERK) signaling pathways which promotes the tumor progression [18-20]. In HCC model, Lai et al. found that Hep3B with overexpressed SULF-2 promoted the proliferation and migration. Furthermore, this study revealed SULF-2 upregulated in human HCC tissues compared to the adjacent tissue for 57% and the patients with high level of SULF-2 had worse prognosis than those who had low level of SULF-2 [20]. These previous studies provided the possibility of SULF-2 to become a candidate biomarker for HCC. Therefore, this study was aimed at evaluating the clinical application of serum SULF-2 in patients with HBV-related HCC compared to patients with non-HCC and healthy controls.

Methodology

Subjects and blood sample collection

Serum samples for the measurement of SULF-2 levels were obtained from patients who were diagnosed with HBV-related HCC for the first time at King Chulalongkorn Memorial Hospital. The diagnosis of HCC was based on standard criteria of typical imaging studies and/or histopathology according to American Association for the Study of Liver Diseases (AASLD) guideline. The control groups comprised 2 groups included patients with chronic HBV infection without evidence of HCC and healthy volunteers from National blood center, Thai Red Cross society.

All of the subjects were informed the aims of the research and signed the consent forms. This research was approved by the Ethics committee, Faculty of Medicine, Chulalongkorn University. The serum samples were separated via centrifugation and stored at -80ºC until use.

Evaluation of circulating SULF-2

The concentration of SULF-2 was quantitated by human SULF-2 enzyme-linked immunosorbent assay (ELISA) kit (Biomartik, USA) according to the manufacturer's instruction. Briefly, 100 microliters of the serum samples diluted by equally volume of Phosphate-buffered saline (PBS) were pipetted to the pre-coated human SULF-2 96 well plate then the plate was incubated at 37 ºC for 1 hour. The detection reagents were added and incubated for 1.30 hours. After that, the plate was washed by wash buffer 5 times before tetramethylbenzidine (TMB) substrate was added and incubated further at 37 ºC for 20 minutes. Finally, the reaction was stopped via hydrogen peroxide stop solution and the plate was measured the optical density at 450 nm by microplate reader.

Statistical analysis

Data were expressed as percentage, mean and standard deviation as appropriate. Comparisons between groups were analyzed by the χ^2 or Fisher's exact test for categorical variables and by the Mann-Whitney test or Student's t test for quantitative variables. Receiver-operating characteristics (ROC) curves were used to evaluate the diagnostic performance of the serum markers in discriminating HCC from the other groups. Pearson and Spearman correlation coefficient were applied for correlation between serum SULF-2 and other parameters. The analysis of overall survival of patients with HCC was calculated by the Kaplan-Meier method using the log-rank test. The Cox regression analysis was assessed to identify factors associated with overall survival. *P* values <0.05 were considered statistically significant. All statistical analyses were performed using the SPSS software for windows 22.0 (SPSS Inc., Chicago, IL).

Results and discussion

Clinical characteristics of subjects

In this study, there were 146 and 119 patients with HCC and non-HCC, respectively. In addition, 50 individuals were recruited as healthy controls. Table 1 represented the baseline characteristics of subjects in this study. The HCC group showed older mean age and higher of male ratio compared with the non-HCC group and healthy controls ($P<0.001$). The levels of TB, AST, ALT, ALP and AFP in the HCC group were significantly increased compared to the non-HCC group ($P<0.001$), whereas mean serum albumin level was lower in the HCC group ($P<0.001$).

SULF-2 level significantly increased in the HCC group

The serum levels of SULF-2 in all subjects were measured by the ELISA method. The mean serum SULF-2 level in the HCC group was significantly higher than the non-HCC group (27.3 ± 10.3 vs.18.5 ± 5.2 ng/ml) and healthy controls (15.8 ± 4.3 ng/ml, $P<0.001$). Likewise, the mean level of this marker in the non-HCC group was significantly higher than healthy controls ($P<0.001$) (Figure 1). The ROC curves for SULF-2 and AFP were then generated. The area under the curve (AUROC) in differentiating HCC from the other groups was 0.79 [95 % confidence interval (CI); 0.73-0.85, $P<0.001$] for SULF-2 and 0.90 (95%CI; 0.86-0.94, $P<0.001$) for AFP (Figure 2).

To our knowledge, this is the first report that examines the serum level of SULF-2 in patients with HCC. Our data clearly demonstrated that patients with HCC exhibited significantly higher of serum SULF-2 than patients without HCC, suggesting that this protein might represent a novel marker of HCC in patients with chronic HBV infection. In agreement with our report, previous data showed that SULF-2 mRNA was upregulated within HCC tissue compared to its adjacent liver tissue, which might activate several pathways leading to the growth of tumor [20]. Our findings were also in line with other reports including head and neck, breast and lung cancers[21-23].

Table 1 Baseline characteristics of subjects in this study. The data was shown in mean ± SD, n (%). Total bilirubin, TB; Aspartate transaminase, AST; Alanine aminotransferase, ALT; Alpha-fetoprotein, AFP; Barcelona clinic liver cancer, BCLC.

Characteristic	Healthy Controls (n=50)	Non-HCC (n=119)	HCC (n=146)	*P-value*
Sex				<0.001
Male	27 (54)	66 (55)	116 (79)	
Female	23 (46)	53 (45)	30 (21)	
Age (years)	48.7 ± 4.9	53.2 ± 13.0	59.2 ± 10.7	<0.001
TB (mg/dL)		1.0 ± 1.6	1.1 ± 0.8	<0.001
Albumin (g/dL)		4.8 ± 5.1	3.5 ± 0.6	<0.001
AST (U/L)		34.1 ± 27.9	97.6 ± 101.0	<0.001
ALT (U/L)		44.5 ± 47.6	62.9 ± 52.5	<0.001
AFP (ng/ml)		6.0 ± 12.4	31,952 ± 128,614	<0.001
Tumor size (cm)			9.0 ± 5.9	
Child-Pugh A/B-C/unknown			97 (66.4)/26 (17.8)/23 (15.8)	
BCLC stage 0-A/B/C-D			31 (21.2)/75 (51.4)/40 (27.4)	

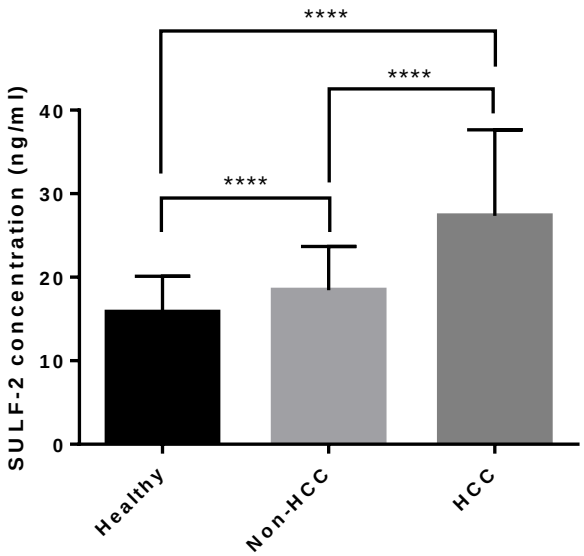

Figure 1 The serum SULF-2 levels in studied groups. Patients with HCC had the highest SULF-2 concentration (27.3 ± 10.3 ng/ml) compared to the non-HCC group (18.5 ± 5.2 ng/ml) and healthy controls (15.8 ± 4.3 ng/ml, *P*<0.001).

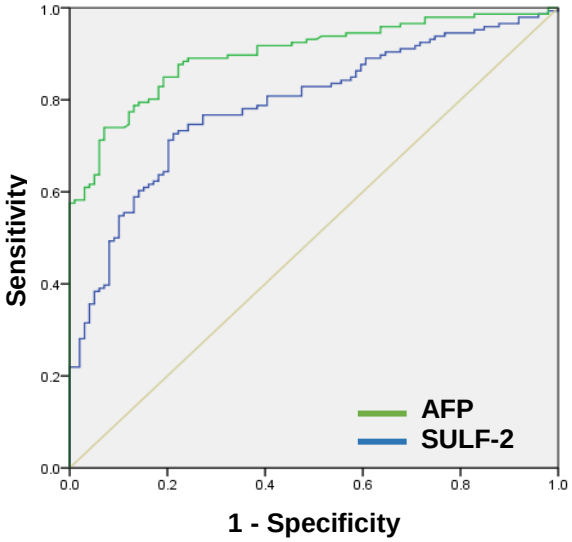

Figure 2 The ROC curve was determined to identify the cut-off point of SULF-2 which was 25 ng/ml.

SULF-2 levels positively correlated with clinical parameters

The correlation of SULF-2 levels with clinical parameters in the HCC group was further analyzed. There was a positive correlation between serum SULF-2 and AFP concentrations as shown in Figure 3A (r=0.286, *P*=0.001). Serum SULF-2 levels were also positively associated with tumor size (r=0.277, *P*=0.001) as demonstrated in Figure 3B. Moreover, serum SULF-2 correlated with Child-Pugh classification (r=0.206, *P*=0.016) and tumor stage (BCLC stage) (r=0.274, *P*=0.001). These data suggested that SULF-2 levels were positively correlated with the progression and severity of HCC.

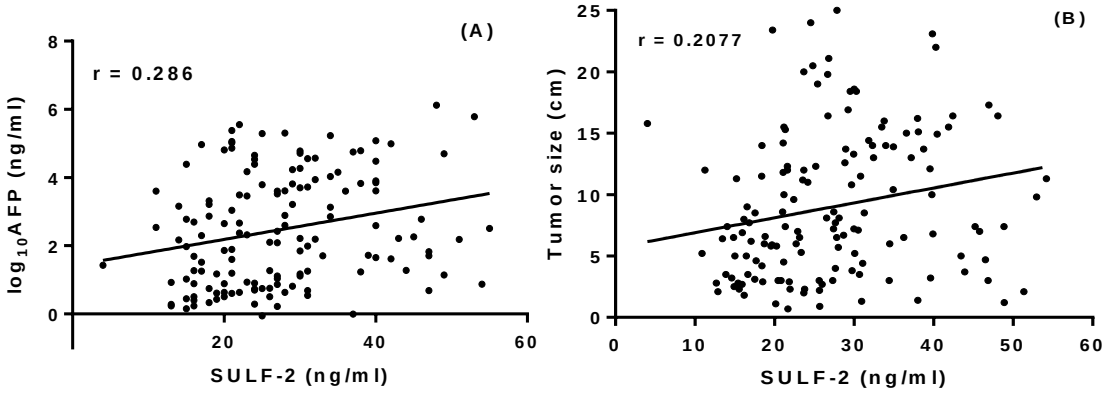

Figure 3 The positive correlation of SULF-2 and clinical parameters. (A) Serum AFP (B) Tumor size

Serum SULF-2 as a prognostic marker for HCC

To evaluate the association between serum SULF-2 levels and overall survival of HCC, patients with HCC were divided into two groups based on the median value of the marker (25 ng/ml). The overall survival of patients with low SULF-2 levels (<25 ng/ml) was significantly better than that of patients whose serum levels were ≥ 25 ng/ml (*P*<0.001 by log rank test) (Figure 4).

Serum SULF-2 level was entered into the multivariate analysis together with other variables that might influence prognosis. These factors included age, gender, serum TB, albumin, ALT, AFP level, tumor size, Child-Pugh score and BCLC stage. The multivariate analysis using the Cox proportional hazards model revealed that high serum SULF-2 and tumor size were prognostic factors of overall survival in patients with HCC (Table 2).

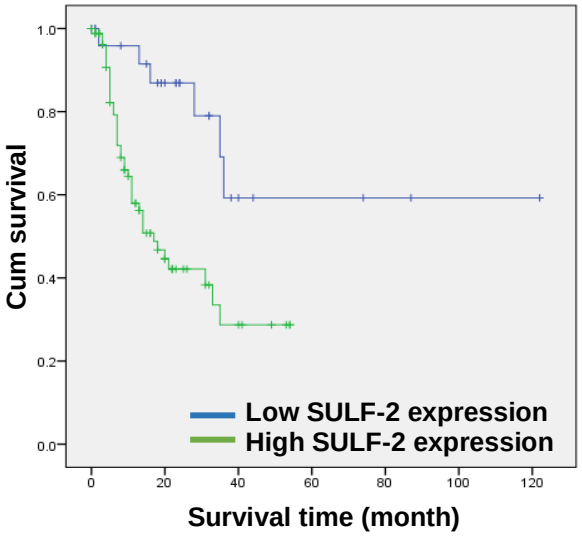

Figure 4 The Kaplan-Meier survival curve suggested that patients with high SULF-2 was associated with poor overall survival (*P*<0.001).

Table 2 The univariate and multivariate analysis. Total bilirubin, TB; Alanine aminotransferase, ALT; Alpha-fetoprotein, AFP; Barcelona clinic liver cancer, BCLC.

Factor	Categories	The HCC group			
		Univariate analysis		Multivariate analysis	
		OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Sex	Male or Female	0.798 (0.384-1.658)	0.545		
Age (years)	<60 or ≥60	0.656 (0.365-1.181)	0.160		
TB (mg/dL)	<0.99 or ≥0.99	1.004 (0.562-1.793)	0.990		
Albumin (g/dL)	<3.55 or ≥3.55	0.710 (0.383-1.317)	0.277		
ALT (U/mL)	<48 or ≥48	2.056 (1.135-3.724)	0.017	1.346 (0.724-2.502)	0.348
AFP (ng/mL)	<162.65 or ≥162.65	2.675 (1.436-4.984)	0.002	1.836 (0.924-3.646)	0.083
SULF-2 (ng/mL)	<25 or ≥25	3.488 (1.758-6.816)	<0.001	3.278 (1.604-6.701)	0.001
Tumor size (cm)	<7.40 or ≥ 7.40	4.818 (2.485-9.342)	<0.001	3.391 (1.515-7.588)	0.003
Child-Pugh	A or B-C	1.825 (0.897-3.713)	0.097		
BCLC stage	0-B or C-D	3.381 (1.840-6.215)	<0.001	1.318 (0.655-2.654)	0.439

Conclusion

Patients with HCC had the highest level of SULF-2 compared with the non-HCC group and healthy controls, suggesting that SULF-2 could be a novel diagnostic marker of HCC. In addition, significant correlations of serum SULF-2 levels with tumor size and stage were found, which indicates that this marker might play an important role in promoting HCC progression. Finally, our results also showed that serum SULF-2 could serve as a prognostic marker for patients with HBV-related HCC.

References

1. de Lope CR, Tremosini S, Forner A, Reig M, Bruix J. J Hepatol. 2012;56:S75-S87.
2. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. CA Cancer J Clin. 2015;65:87-108.
3. Chonprasertsuk S, Vilaichone RK. Jpn J Clin Oncol. 2017;47:294-297.
4. Ghouri YA, Mian I, Rowe JH. J Carcinog. 2017;16.
5. Wanich VR, Chotivitayatarakorn P, Siramolpiwat S. Asian Pac J Cancer Prev.2016;17.
6. Zhu RX, Seto WK, Lai CL, Yuen MF. Gut Liver. 2016;10:332-339.
7. Kremsdorf D, Soussan P, Paterlini BP, Brechot C. Oncogene. 2006;25:3823.
8. Lin S, Hoffmann K, Schemmer P. Liver Cancer. 2012;1:144-158.
9. Saraswat VA, Pandey G and Shetty S. J Clin Exp Hepatol. 2014;4:S80-S89.
10. Forner A, Gilabert M, Bruix J and Raoul JL. Nat. Rev. Clin. Oncol. 2014;11:525.
11. Bai DS, Zhang C, Chen P, Jin SJ, Jiang GQ. Sci. Rep. 2017;7:12870.
12. Randeep AJ, Michael N, Brian C, Jackie BH, Jorge R, John F. Dig Dis Sci. 2001;46.
13. Sherman M, Peltekian KM, Lee C. J Hepatol. 2005;22:432-438.
14. Zhao Y-J, Ju Q, Li G-C. Mol Clin Oncol. 2013;1:593-598.
15. Zheng X, Gai X, Han S, Moser CD, Hu C, Shire AM, Floyd RA, Roberts LR. Genes Chromosomes Cancer. 2012;52:225-236.
16. Al-Gayyar MH, Abbas A, Hamdan AM. Biological Chemistry. 2016;397:257.
17. Rosen SD, Lemjabbar AH, Expert Opin Ther Targets. 2010;14:935-949.
18. Lai JP, Oseini AM, Moser CD, Yu C, Elsawa SF, Hu C, Nakamura I, Han T, Aderca I, Isomoto H, Garrity-Park MM, Shire AM, Li J, Sanderson SO, Adjei AA, Fernandez-Zapico ME, Roberts LR. J Hepatol. 2010;52:1680-1689.
19. Chen G, Nakamura I, Dhanasekaran R, Iguchi E, Tolosa EJ, Romecin PA, Vera RE, Almada LL, Miamen AG, Chaiteerakij R, Zhou M, Asiedu MK, Moser CD, Han S, Hu C, Banini BA, Oseini AM, Chen Y, Fang Y, Yang D, Shaleh HM, Wang S, Wu D, Song T, Lee J-S, Thorgeirsson SS, Chevet E, Shah VH, Fernandez-Zapico ME, Roberts LR. Cancer Research. 2017;77:632.
20. Lai JP, Sandhu DS, Yu C, Han T, Moser CD, Jackson KK, Guerrero RB, Aderca I, Isomoto H, Garrity-Park MM, Zou H, Shire AM, Nagorney DM, Sanderson SO, Adjei AA, Lee JS, Thorgeirsson SS, Roberts LR. J Hepatol. 2008;47:1211-1222.
21. Lui NS, Yang Y-W, van Zante A, Buchanan P, Jablons DM, Lemjabbar-Alaoui H. Plos one. 2016;11:e0148911.
22. Morimoto-Tomita M, Uchimura K, Bistrup A, Lum DH, Egeblad M, Boudreau N, Werb Z, Rosen SD. Neoplasia. 2005;7:1001-1010.
23. Flowers SA, Zhou X, Wu J, Wang Y, Makambi K, Kallakury BV, Singer MS, Rosen SD, Davidson B, Goldman R. Oncotarget. 2016;7:43177-43187.

Acknowledgement

This study was supported by the Center of Excellence in Hepatitis and Liver Cancer, Department of Biochemistry, Faculty of Medicine, Chulalongkorn University and Chulalongkorn university graduate scholarship to commemorate the 72nd anniversary of his Majesty King Bhumibol Adulyadej.