ORIGINAL ARTICLE

Role of superoxide dismutase in hepatitis B virus-related hepatocellular carcinoma

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Background: Reactive oxygen species (ROS) play important roles in hepatocarcinogenesis. Superoxide dismutase (SOD) is involved in the repair of ROS. Serum alpha-fetoprotein (AFP) is the "golden marker" for diagnosing hepatocellular carcinoma (HCC), and one major shortcoming of its use is that it is insensitive for the early detection of HCC. Therefore, we evaluated serum SOD levels and their association with AFP in hepatitis B virus (HBV)-related HCC. **Materials and Methods:** A total of 279 subjects were divided into three groups: 99 HBV patients with HCC, 73 HBV patients without HCC, and 107 sex- and age-matched healthy controls. Serum levels of SOD were assayed using colorimetry, while AFP levels were measured by electrochemiluminescence immunoassay. **Results:** A highly significant elevation was found in AFP in HBV-with HCC patients compared to HBV-without HCC patients and control subjects ($P < 0.001$). Alternatively, serum SOD levels were significantly decreased in patients with HCC compared to HBV patients without HCC and healthy controls ($P < 0.001$). Furthermore, serum SOD was negatively correlated with AFP ($r = -0.505$, $P < 0.001$) in HBV-with HCC patients. **Conclusion:** SOD and AFP might be simultaneously evaluated to improve the HCC detection rate.

Key words: Alpha-fetoprotein, hepatitis B virus, hepatocellular carcinoma, serum, superoxide dismutase

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most frequently diagnosed cancer and the second leading cause of cancer deaths worldwide.[1] According to a 2012 report from Torre *et al.*,[1] an estimated 782,500 new liver cancer cases and 745,500 deaths occurred worldwide, and China alone accounted for about 50% of these cases and deaths. It is well-known that HCC is a complex and multi-factorial process, and its etiology still remains elusive.[2] Major risk factors for HCC include chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections, aflatoxin exposure, Type 2 diabetes, cirrhosis related to heavy alcohol consumption, nonalcoholic fatty liver disease (associated with obesity), and smoking.[1,3-5] In general, approximately 75–85% of HCC patients are attributable to chronic HBV infections, especially in less developed countries such as China.[6,7]

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Early detection of HCC is the most critical factor for increasing patient survival rates.[8] The most widely used tumor marker for HCC is serum alpha-fetoprotein (AFP), which is considered the "gold standard" for HCC detection.[9] However, serum AFP is insensitive for early HCC detection in clinical practice, and it may also be elevated in benign chronic liver diseases such as chronic viral hepatitis and liver cirrhosis without HCC.[9,10] Based on these shortcomings, it is clear that there is a critical need for newer markers to complement AFP measurements, specifically, markers that will enable an HCC diagnosed in the early tumor stage.[11]

Oxidative stress has been shown to play an important role in the pathogenesis of HCC.[12] Oxidative stress can cause an imbalanced antioxidant defense system and an enhanced production of reactive oxygen species (ROS).[13,14] ROS can induce severe DNA damage.[15] These DNA alterations, which activate proto-oncogenes and inactivate tumor suppressor genes, could contribute to the induction, promotion

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and progression of cancer in the oxidative stress affected organs.[16] Superoxide dismutase (SOD) is one of the key components of the antioxidant defense system.[17,18] Among the antioxidant defense systems, SOD is the first and most important line of enzymatic defense against oxidative stress, particularly oxygen free radicals.[19,20] Previous findings have demonstrated that the SOD activity levels were decreased in children with chronic viral hepatitis.[21] Alternatively, Chen *et al.* have shown that serum SOD levels were increased in chronic viral hepatitis after standard medical treatment.[20] Another study by Yahya *et al.*[22] found that there was a significant SOD decrease in HCV-related HCC patients compared to healthy controls. These observations suggest that SOD might play an important role in inhibiting HCC occurrence.

To date, no studies have assessed the association between serum SOD levels and AFP in HBV-related HCC patients. In this study, we aimed to examine serum the SOD level and its association with AFP in HBV-related HCC patients living in Guangxi, China.

MATERIALS AND METHODS

Study population

This retrospective cohort study included 279 subjects with a mean age of 45.42 ± 10.69 years (range 18–76 years). All were divided into three groups: 99 HBV patients with HCC, 73 HBV patients without HCC, and 107 sex- and age-matched healthy controls. The study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University, Guangxi, China, and the methods were carried out in accordance with the approved guidelines. All study subjects provided written informed consent. The informed consent process was conducted by Xiaolian Zhang and Yu Lu. All the contents of the informed consent including background for the need of the study, the aim of study, subjects' rights and duties, who was performing the study, how the study was to be performed were explained to the participants.

The cases were consecutively recruited for this study from January 2015 to June 2015. All HBV-related patients (including with HCC and without HCC) were HBV surface antigen-positive, HBV core antibody positive, hepatitis B e-antigen-positive, or hepatitis B e-antibody-positive for at least 6 months. The HBV patients with HCC were diagnosed based on pathological confirmation combined with at least one positive liver image on computed tomography, magnetic resonance imaging, or ultrasonography, sometimes combined with elevated serum AFP levels (>400 ng/mL).[23,24] We only included the newly diagnosed HCC patients without any treatment. Patients were excluded if they had other hepatitis virus infections (e.g., hepatitis A/C/D/E virus) or a family history of HCC or other malignant neoplasias.

The healthy controls who were negative for HBV markers underwent a general health check-up at the same hospital during the same time frame. They were matched with cases by sex and age. The controls who had cancer or other serious illness, or a family history of cancer or other serious illness were excluded from this study.

Laboratory methods

The participants were fasted overnight before blood collections. The participants sat for at least 15 min before blood was collected. A volume of 6 mL of venous whole blood was collected from each subject and placed into serum gel tubes (Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA). Samples were allowed to stand for 20 min at 4°C, centrifuged at 2500 g for 20 min at 4°C (Anhui USTC Zonkia Scientific Instrument Co., Ltd, Anhui, China) and stored at 4°C until analysis.

Serum AFP levels were determined by an electrochemiluminescence immunoassay using a Cobas® 6000 system E601 (Elecsys module) immunoassay analyzer (Roche Diagnostics, GmbH, Mannheim, Germany). SOD concentrations were measured by colorimetry with a Hitachi 7600-020 analyzer (Hitachi Corp, Tokyo, Japan). All assays were conducted according to the manufacturer's instructions by the same operator using the same batch reagent.

Statistical analysis

Shapiro-Wilk and D'Agostino tests were used to test the normality of data distribution. Nonnormally distributed variables were presented as a median and interquartile range (IQR). If data distribution is nonnormal, differences among groups were compared by nonparametric Kruskal–Wallis *H* test. Demographic characteristics among the three groups were calculated with a χ^2 test for categorical variables. The correlation between different parameters was done using the Spearman's correlation test. A two-tailed $P < 0.05$ was considered to be statistically significant. All statistical analyses were carried out with statistical software SPSS version 16.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Characteristics of the study population

Demographic and clinical parameters of all participants enrolled in this study are presented in Table 1. There was no significant difference for sex among the three groups ($P > 0.05$). The levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (T-Bil), direct bilirubin (D-Bil), gamma-glutamyltransferase (GGT), total

Table 1: Demographic and clinical parameters of the subjects enrolled in this study

Parameters	Controls	HBV patients without HCC	HBV patients with HCC	*P*
Total number	107	73	99	
Demographic parameters				
Sex (male/female)	85/22	55/18	85/14	0.208$^{\Delta}$
Clinical parameters (median [IQR])				
AST (IU/L)	21 (18-23)	43 (28-94)	47 (31-89)	<0.001*
ALT (IU/L)	20 (15-26)	40 (26-73)	45 (30-63)	<0.001*
GGT (IU/L)	25 (17-39)	52 (29-128)	90 (61-160)	<0.001*
ALP (IU/L)	64 (55-78)	80 (54-100)	77 (62-115)	<0.001*
T-Bil (μmol/L)	15.9 (13.0-18.6)	18.2 (12.9-38.4)	18.9 (14.0-25.2)	0.020*
D-Bil (μmol/L)	4.5 (3.5-5.4)	6.6 (3.9-16.5)	7.2 (4.7-9.5)	<0.001*
TBA (μmol/L)	3.7 (2.6-6.0)	12.0 (3.1-61.6)	11.7 (5.1-27.7)	<0.001*
TP (g/L)	77.1 (74.5.0-79.7)	72.8 (69.4-77.7)	65.8 (61.1-71.8)	<0.001*
ALB (g/L)	47.4 (45.5-48.3)	42.6 (37.4-44.6)	36.1 (31.8-40.5)	<0.001*
PA (mg/L)	329.0 (298.9-371.4)	180.0 (111.0-242.8)	129.0 (94.7-170.8)	<0.001*
HBV-DNA, log10 (IU/mL)	No data	4.0 (2.7-5.5)	3.2 (2.7-4.6)	0.028*

$^{\Delta}$*P* value of Chi-square test; **P* value of Kruskal–Wallis *H* test. HBV = Hepatitis B virus; HCC = Hepatocellular carcinoma; IQR = Interquartile range; AST = Aspartate aminotransferase; ALT = Alanine aminotransferase; GGT = Gamma-glutamyltransferase; ALP = Alkaline phosphatase; T-Bil = Total bilirubin; D-Bil = Direct bilirubin; TBA = Total bile acid; TP = Total protein; ALB = Albumin; PA = Prealbumin

protein (TP), albumin (ALB), alkaline phosphatase (ALP), total bile acid (TBA), prealbumin (PA), and HBV-DNA among different groups were abnormally distributed and they were reported as median and IQR. The values of AST, ALT, T-Bil, D-Bil, GGT, ALP, and TBA were significantly increased in HBV-infected patients compared with healthy controls ($P < 0.001$). On the other hand, the levels of TP, ALB, and PA were significantly decreased in HBV-infected patients compared with control subjects ($P < 0.001$). Furthermore, the HBV patients without HCC had a higher HBV-DNA viral load than the HBV patients with HCC ($P < 0.05$).

Serum superoxide dismutase and alpha-fetoprotein levels

We detected and compared serum SOD and AFP levels from 107 controls, 73 HBV-without HCC cases, and 99 HBV-with HCC cases. Serum SOD and AFP levels among different groups were abnormally distributed, and they were reported as median and IQR. Table 2 shows that serum SOD levels were significantly decreased in HBV-with HCC patients when compared to HBV-without HCC patients and healthy controls ($P < 0.001$). There was a highly significant increase for AFP in HCC patients when compared to the HBV-without HCC group and control group ($P < 0.001$).

Correlation between levels of superoxide dismutase and alpha-fetoprotein in hepatitis B virus with hepatocellular carcinoma patients

As shown in Figure 1, serum SOD levels exhibited a negative correlation with AFP levels ($r = -0.505$, $P < 0.001$).

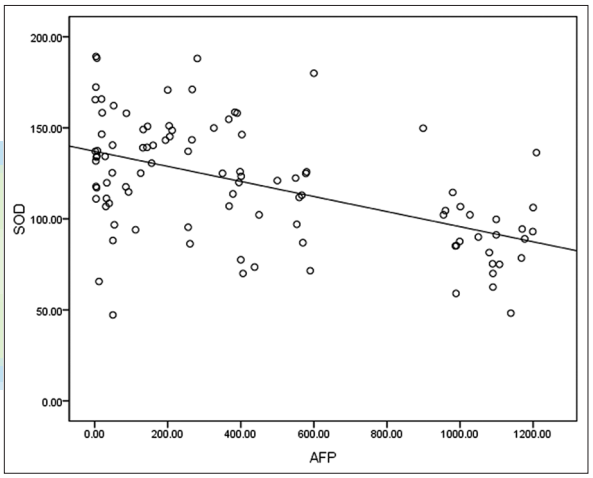

Figure 1: Correlation between serum level of superoxide dismutase and alpha-fetoprotein in hepatitis B virus-related hepatocellular carcinoma group

DISCUSSION

HCC is one of the most common cancers worldwide, with a very poor prognosis, rendering it the second highest cause of cancer-related deaths.[1] It has been suggested that HCC is a complex disease for which the underlying cause remains indistinct. Oxidative stress has been reported to be implicated in the etiology and pathology of HCC.[25] Oxidative stress can lead to an increase in ROS levels and induced DNA fragmentation.[26] Among the antioxidant defense systems, SOD is the most important antioxidant enzyme to prevent cellular injury from ROS.[19] Therefore, we hypothesize that SOD plays a significant role in protecting cells against severe oxidative stress, and thus, preventing the occurrence of HCC.

In the current study, we assessed serum SOD levels in 107 healthy controls, 73 HBV-without HCC patients, and 99 HBV-with HCC patients, and its association with AFP in HBV patients with HCC. To the best of our knowledge, this is the first study to report serum SOD levels and their correlation with AFP in HBV-with HCC patients. The

Table 2: Serum levels of superoxide dismutase and alpha-fetoprotein in hepatitis B virus patients with and without hepatocellular carcinoma and healthy controls

Parameters	Controls	HBV patients without HCC	HBV patients with HCC	*P*
SOD (U/mL)	172.7 (160.4-190.5)	153.0 (128.3-182.3)	119.8 (94.0-143.4)	<0.001*
AFP (ng/mL)	2.2 (1.3-3.0)	3.7 (2.4-15.4)	326.3 (50.0-899.0)	<0.001*

**P* value of Kruskal–Wallis *H* test. SOD = Superoxide dismutase; AFP = Alpha fetoprotein; HBV = Hepatitis B virus; HCC = Hepatocellular carcinoma

results suggest that SOD concentrations in HCC cases were lower than HBV-without HCC patients and control subjects ($P < 0.001$). SOD was also negatively correlated with AFP in HBV-related HCC patients ($r = -0.505$, $P < 0.001$). The decrease in SOD was possibly due to an overutilization of SOD to scavenge ROS or decrease synthesis capacity of SOD in the livers of HCC patients. In general, these findings support our hypothesis that SOD levels play a vital role in the prevention of HCC and imply that decreased SOD levels might be a potential biomarker in the early diagnosis of HCC.

Due to the critical role of SOD in an anti-tumor role, serum SOD levels in liver diseases with viral hepatitis have been extensively studied. In 2000, Chrobot *et al.* assessed 100 children with chronic HBV and/or HCV infections and found a significant decrease in SOD activity in children with chronic hepatitis B and C.[21] Osman *et al*. examined 130 patients with viral hepatitis and observed that serum SOD activity was significantly decreased in the patient group as compared to the control group.[27] Another study was conducted by Chen *et al.* in 2013 and included thirty patients with chronic viral hepatitis;[20] this group reported that the SOD levels were significantly increased after standard medical treatment. Yahya *et al.* compared forty HCV-with HCC patients and twenty patients with HCV and found serum SOD levels were significantly decreased in HCC patients compared to control subjects.[22] However, these studies were both performed with small sample sizes and only investigated serum SOD levels. In this study, we investigated serum SOD levels in a larger sample size (99 HBV patients with HCC, 73 HBV patients without HCC, and 107 healthy controls). Our results indicate the serum SOD levels were significantly decreased in HCC patients compared to HBV patients without HCC and controls. With a larger total number of subjects, more robust results were obtained in the current study than in previous studies.

Serum AFP is the "golden marker" for diagnosing HCC; however, one major shortcoming of its use is that it is insensitive for the early detection of HCC.[28] Therefore, an additional marker, such as SOD, has been suggested to be simultaneously measured to improve the HCC detection rate. This study showed a significant increase in serum AFP in HCC patients compared to HBV patients without HCC and healthy controls. Furthermore, we evaluated the association of serum SOD and AFP in HBV-with HCC patients and revealed a negative correlation between them.

CONCLUSION

The serum antioxidant of SOD was significantly correlated with AFP in HBV patients with HCC; thus, it might be a potential biomarker in the early diagnosis of HCC. SOD and AFP levels might be simultaneously evaluated to improve the HCC detection rate.

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Conflicts of interest
There are no conflicts of interest.

AUTHORS' CONTRIBUTION

XQ and ShL contributed in the conception of the work, conducting the study, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. ChR and DY performed the experiments, analyzed the data, revised the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. XZ and YL contributed in drafting and revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work.

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