ORIGINAL ARTICLE

Diagnostic Value of Serum DNASE1L3 in Hepatitis B Virus-Related Hepatocellular Carcinoma

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SUMMARY

Background: Deoxyribonuclease 1-like 3 (DNASE1L3) is an endonuclease associated with many autoimmune diseases and tumors. However, the serum DNASE1L3 level in hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC) remains unreported. Thus, this study compared the diagnostic value of DNASE1L3 and alpha-fetoprotein (AFP) individually and in combination in HBV-related HCC.

Methods: The study population consisted of 88 patients with HBV-related HCC, 80 patients with HBV-related liver cirrhosis (LC) and 88 control subjects. The serum DNASE1L3 levels were measured using an enzyme-linked immunosorbent assay. The serum AFP was also assayed.

Results: Our data showed that the serum DNASE1L3 levels were significantly higher in patients with HBV-related HCC than in the healthy controls and patients with LC. When the two biomarkers were analyzed individually, the receiver operating characteristic curve analysis showed that the areas under the curve of DNASE1L3 and AFP were 0.898 and 0.866, respectively. When DNASE1L3 and AFP were combined, the area under the curve was 0.951. The sensitivities of DNASE1L3 and AFP were 72.73% and 74.81%, respectively, and the specificities were 93.18% and 92.05%, respectively, in the diagnosis of HBV-related HCC. The sensitivity of the two combined could be improved to 89.77%. However, no correlation was found between serum DNASE1L3 and AFP in HBV-related HCC patients (r = 0.005, p = 0.734).

Conclusions: Serum DNASE1L3 has high sensitivity and specificity in the diagnosis of HCC. DNASE1L3 combined with AFP has higher sensitivity and can improve the diagnostic efficiency of HBV-related HCC.


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KEY WORDS
deoxyribonuclease 1-like 3, hepatocellular carcinoma, alpha-fetoprotein, diagnosis

INTRODUCTION

Hepatocellular carcinoma (HCC) is a common type of liver cancer with high morbidity and mortality. Hepatitis B virus (HBV) infection is a very common blood-borne liver disease that has been well-established as the key etiology in end-stage liver diseases and HCC. It is one of the most important risk factors for the development, progression, and recurrence of HCC after treatment [1,2]. Several methods are available for HCC di-
agnosis. The recommended non-invasive methods include imaging techniques, such as magnetic resonance imaging, and the use of tumor markers. Currently, the laboratory diagnosis of HCC relies mainly on the detection of patients with a serum alpha-fetoprotein (AFP) level. Although the AFP value for diagnosing HCC has already been established, it is more easily influenced by other factors, and there is a 20% - 30% or even higher rate of false positives or false negatives. Sometimes, there is no abnormal increase even in the late stage of HCC, thus reflecting the low sensitivity of serum AFP. Other studies have shown that the serum AFP level is not related to the stage and prognosis of HCC [3]. To improve the diagnostic performance of HCC, more reliable serum biomarkers need to be identified.

Deoxyribonuclease 1-like 3 (DNASE1L3) is an endonuclease that is produced by dendritic cells and macrophages and degrades the chromatin of apoptotic or necrotic cells [4,5]. The level of DNASE1L3 is related to a variety of autoimmune diseases [6,7] and tumors [8]. Recently, Wang et al. found DNASE1L3 was downregulated in both mRNA and protein levels in HCC tissues [9]. However, the serum DNASE1L3 levels in HBV-related HCC and liver cirrhosis (LC) patients have not yet been reported. Thus, we detected the concentration of serum DNASE1L3 in HBV-related HCC, HBV-related LC patients, and healthy controls and further investigated the diagnostic value of serum DNASE1L3 in detecting HBV-related HCC. This was achieved by comparing the sensitivity, specificity, and receiver operating characteristics (ROCs) of the biomarkers individually and in combination among HBV-related HCC patients.

MATERIALS AND METHODS

Study design and participants
A total of 256 participants aged 28 - 76 years were included in the study. All participants were recruited between January 2017 and November 2018 at the Department of Spleen and Stomach, the Third Affiliated Hospital of Guangxi University of Chinese Medicine in Liuzhou, China. Among the patients, 88 were assigned to the HBV-related HCC group and 80 to the LC group. Another 88 individuals who underwent physical examination. The exclusion criteria were co-infection with hepatitis A/C/D/E viruses and having other liver diseases, such as alcoholic liver diseases or autoimmune hepatitis.

The study protocol was approved by the ethics committee of the Third Affiliated Hospital of Guangxi University of Chinese Medicine and adhered to the Declaration of Helsinki. All participants provided written informed consent.

Laboratory tests
Vacuum serum tubes were used to collect 2 mL of venous blood from all participants after 12 hours of fasting. The tubes were centrifuged at 3,000 rpm for 10 minutes to obtain the serum. The serum samples were frozen at -20°C within 8 hours of collection and then transferred to a -80°C environment for long-term storage. The samples were thawed at 4°C prior to assay performance. Serum DNASE1L3 was measured by an enzyme-linked immunosorbent assay using a quantitative test kit (Cusabio, Wuhan, China). The intra-assay and inter-assay coefficients of variation were less than 10.0%. Serum ALT, AST, TBIL, and ALB were tested with a Hitachi 7600 automatic biochemical analyzer (Hitachi Co., Tokyo, Japan). Serum AFP was measured with the Cobas e601 system.

Statistical analysis
Statistical analysis was conducted using SPSS 22.0 software (IBM Corporation, Armonk, NY, USA) and GraphPad Prism 7 (GraphPad Software Inc., La Jolla, CA, USA). The normal distribution of continuous variables was checked using the Shapiro-Wilk test. Normally distributed variables were presented as the means and standard deviations, and non-normally distributed variables were reported as the median (interquartile range [IQR]). The categorical variables were compared using the chi-square test and Fisher’s exact test. For the continuous variables, an unpaired t-test was used for comparing two groups, and analysis of variance with Bonferroni correction (two-tailed) was used for more than two groups. Pearson’s coefficient (r) was used to examine the correlations between pairs of variables. ROC curve analysis was performed to evaluate the cutoff value of a particular biomarker and its area under the curve (AUC). Significance was set to p < 0.05 for all analyses.

RESULTS

Clinical characteristics of the study population
The demographic and clinical characteristics of HCC patients, LC patients, and healthy controls are summarized in Table 1. No significant difference was found in age and gender composition between the case group and the control group. In testing the laboratory parameters, such as AFP, TP, ALB, ALT, and AST, significant differences were observed between each group (p < 0.001).
Table 1. Clinical characteristics of patients and controls.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control (n = 88)</th>
<th>LC (n = 80)</th>
<th>HCC (n = 88)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>48.7 ± 13.3</td>
<td>54.5 ± 11.1</td>
<td>53.9 ± 11.4</td>
<td>0.194</td>
</tr>
<tr>
<td>Gender (n, %)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>60 (68.18%)</td>
<td>58 (72.50%)</td>
<td>63 (71.59%)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>28 (31.82%)</td>
<td>22 (27.50%)</td>
<td>25 (28.41%)</td>
<td></td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>17.0 (13.0 - 23.0)</td>
<td>34 (22 - 58.75)</td>
<td>38 (24 - 72.75)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>19.0 (16.0 - 26.0)</td>
<td>47.5 (29.25 - 71.75)</td>
<td>51 (29 - 133.3)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>TBIL (μmol/L)</td>
<td>12.35 (10.23 - 15.3)</td>
<td>25.25 (15.3 - 55.95)</td>
<td>23.95 (15.53 - 55.95)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>DBIL (μmol/L)</td>
<td>5.1 (3.65 - 5.8)</td>
<td>5.9 (4.23 - 29.95)</td>
<td>6.5 (4.2 - 24.33)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>TP (g/L)</td>
<td>71.3 (67.08 - 76.5)</td>
<td>68.5 (64.8 - 75.85)</td>
<td>67.5 (61.85 - 75.7)</td>
<td>0.006</td>
</tr>
<tr>
<td>ALB (g/L)</td>
<td>39.95 (38.53 - 45.15)</td>
<td>35.2 (31.5 - 40.58)</td>
<td>35.1 (29.73 - 40.28)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>AFP (ng/mL)</td>
<td>2.48 (1.8 - 3.31)</td>
<td>2.77 (2.18 - 5.57)</td>
<td>229.5 (6.723 - 7,098.00)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Abbreviations: ALT - alanine aminotransferase, AST - aspartate aminotransferase, TBIL - total direct and indirect bilirubin, DBIL - direct bilirubin, TP - total protein, ALB - albumin.

Table 2. Associations of DNASE1L3 expression with clinical characteristics in HCC patients.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (U/L)</td>
<td>0.117</td>
<td>0.276</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>0.174</td>
<td>0.106</td>
</tr>
<tr>
<td>TBIL (μmol/L)</td>
<td>0.075</td>
<td>0.483</td>
</tr>
<tr>
<td>DBIL (μmol/L)</td>
<td>0.102</td>
<td>0.344</td>
</tr>
<tr>
<td>TP (g/L)</td>
<td>-0.110</td>
<td>0.304</td>
</tr>
<tr>
<td>ALB (g/L)</td>
<td>-0.208</td>
<td>0.051</td>
</tr>
<tr>
<td>AFP (ng/mL)</td>
<td>0.140</td>
<td>0.193</td>
</tr>
</tbody>
</table>

Table 3. ROC analyses of the diagnostic values of the two indexes alone and their combinations for distinguishing HCC group from control group.

<table>
<thead>
<tr>
<th>Index</th>
<th>AUC</th>
<th>p</th>
<th>Cutoff</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Youden index</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNASE1L3</td>
<td>0.871</td>
<td>&lt; 0.001</td>
<td>162.16</td>
<td>71.59</td>
<td>90.91</td>
<td>0.625</td>
</tr>
<tr>
<td>AFP</td>
<td>0.866</td>
<td>&lt; 0.001</td>
<td>5.22</td>
<td>78.41</td>
<td>92.05</td>
<td>0.704</td>
</tr>
<tr>
<td>DNASE1L3-AFP</td>
<td>0.931</td>
<td>&lt; 0.001</td>
<td>0.242</td>
<td>88.64</td>
<td>87.50</td>
<td>0.761</td>
</tr>
</tbody>
</table>

negative HBV-related HCC patients (AFP ≤ 10 ng/mL) accounted for 26.0%, and AFP-positive (AFP > 10 ng/mL) participants in the healthy control group accounted for 3.0%. AFP lacked sensitivity and specificity in the diagnosis of HBV-related HCC.
Figure 1. Serum DNASE1L3 levels in HBV-related HCC, HBV-related LC, and controls.

** - p < 0.001.

Figure 2. ROC analyses of the diagnostic values of the two indexes alone and their combinations for distinguishing HCC group from control group.

**Serum levels of DNASE1L3 in patients with HCC and LC**

As the serum levels of biomarkers are sometimes extremely high, we measured the median and IQR values to avoid these extreme outliers from producing misleading mean values and thereby allow more accurate comparisons. The median concentration of DNASE1L3 in patients with HCC was 309.00 ng/mL (IQR: 121.6 - 785.4), which is significantly higher than that in the healthy controls (30.95 ng/mL, IQR: 17.1 - 90.06, p <
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0.001). The serum DNASE1L3 level in patients with LC was 85.32 ng/mL (IQR: 33.74 - 164.4), which was higher than that in the controls. Compared with the patients with LC, the serum DNASE1L3 levels were significantly higher in patients with HCC (p < 0.001). The differences between the HCC group, LC group, and control group are shown in Figure 1.

Relationship between serum DNASE1L3 levels and clinical factors in HCC patients
The possible association between serum DNASE1L3 concentration and ALT, AST, TBIL, DBIL, TP, ALB, and AFP in the HCC patients was further analyzed. The serum DNASE1L3 level in the HCC patients was not correlated with AFP (r = 0.005, p = 0.734) or any other indicators detected in this study (Table 2).

ROC curve analysis for HCC/LC and DNASE1L3
To evaluate the diagnostic accuracy of the serum DNASE1L3 levels in the HCC patients, a ROC curve analysis was performed (Figure 3). When used to predict HCC, the AUC of the ROC curve of serum DNASE1L3 was 0.871 (p < 0.001, 95% CI = 0.812 - 0.917). The best cutoff value was 162.16 ng/mL, with a sensitivity of 71.59% and a specificity of 90.91%. In comparison, the AUC of AFP to predict HCC was 0.866 (p < 0.001, 95% CI = 0.807 - 0.913), with a sensitivity of 78.41% and a specificity of 92.05%. The AUC of the combination of DNASE1L3 and AFP was 0.931 (p < 0.001, 95% CI = 0.882 - 0.963), with a sensitivity of 88.64% and a specificity of 87.50%. The AUC and sensitivity of DNASE1L3 combined with AFP were significantly higher than that of DNASE1L3 or AFP alone (Figure 2).

DISCUSSION
In this study, we tested serum DNASE1L3 in patients with HCC and LC for the first time and comprehensively evaluated the potential value of serum DNASE1L3 in HCC patients. The results indicated that the serum DNASE1L3 levels were significantly elevated in the HCC patients. The ROC analysis showed that DNASE1L3 combined with AFP had a superior predictive value for HCC and that the sensitivity and AUC were significantly higher than that of DNASE1L3 or AFP alone. However, we found that the serum DNASE1L3 levels in the HCC patients were not correlated with AFP or other indicators.

The diagnosis of liver cancer as early as possible is essential for curative interventions, which improve the prognosis and long-term survival of patients. Cellular immunity plays an important role in promoting tumor growth, progression, and metastasis. As the most predominant tumor-infiltrating immune cells, tumor-associated macrophages (TAMs) mainly express the characteristics of M2 macrophages, which are significant for a novel-identified independent prognostic factor for survival in patients with HCC [10]. DNASE1L3 is produced by dendritic cells and macrophages and is uniquely capable of digesting chromatin in microparticles released from apoptotic cells [4,5]. Chromatin is considered to be the major source of autoantigens that drive the generation of autoantibodies [11,12], which can trigger autoimmune responses. Previous studies have shown that the level and activity of DNASE1L3 were related to a variety of autoimmune diseases and tumors, such as systemic lupus erythematosus [6], dermatomyositis/polymyositis [7], rheumatoid arthritis [8], ankylosing spondylitis [9], type 1 diabetes [14], systemic sclerosis disease [15], and hypocomplementemnetic urticarial vasculitis syndrome [16]. In addition, the DNASE1L3 gene has been found to be overexpressed in early clear cell renal cell carcinoma [17]. TAMs, which are closely related to the secretion of DNASE1L3, are associated with the progression of clear cell renal cell carcinoma [18,19]. This result is similar to that of our current study in terms of the DANSE1L3 level in HCC patients. According to previous research, DNASE1L3 produced from liver macrophages causes the karyolysis of necrotic hepatocytes and degrades the chromatin of apoptotic or necrotic cells [20]. Moreover, DNASE1L3 gene is considered to act as prognosis and progression biomarkers of HCC [21].

Hepatic macrophages can arise either from circulating monocytes or from self-renewing embryo-derived local macrophages called Kupffer cells [22]. In the process of hepatic disease caused by HBV infection, HBV specifically infects hepatocytes and causes immune-mediated liver damage. Long-term infectious injury results in the release of inflammatory cytokines and chemokines and the production of many inflammatory monocyte-derived macrophages [23,24]. Subsequently, an excess of DNA-SE1L3 was produced to digest necrotic and apoptotic hepatocytes. Therefore, it is understandable that chronic high levels of HBV infection can elevate the serum DNASE1L3 levels in patients with LC. However, Wang et al. [9] found DNASE1L3 downregulated in both mRNA and protein levels in HCC tissues, which was contrary to our result; therefore, the concentration of DNASE1L3 in serum is not consistent with the protein levels in HCC tissues. This suggests that the immune system is activated in patients with HCC, causing other tissues to secrete DNASE1L3.

In China, HCC generally arises from a fibrotic or cirrhotic liver caused by infection with HBV. Macrophages are key components in the formation of HCC and are conventionally classified into M1 and M2 subtypes [25]. Recent studies have shown that the levels of M2 macrophages correlate with increased tumor nodules and venous infiltration in HCC patients, promoting tumor growth and invasiveness in HCC [25,26]. Therefore, it is reasonable to believe that the level of macrophages increases with the development of the disease and the resulting increase in DNASE1L3 to digest necrotic and apoptotic hepatocytes. In recent years, AFP-L3 and PIVKA-II, as new diagnos-
tic indicators of HCC, have been gradually applied in clinical practice. Previous studies have shown that combining AFP, AFP-L3, and PIVKA-II improves the diagnostic accuracy for HCC among cirrhotic patients compared with using each marker individually [27,28]. Qi et al. [28] combined the four indicators of AFP, AFP-L3, PIVAKII, and CEA and found that the combined AUCs of AFP, AFP-L3, and PIVAKII showed the highest value compared with the AUCs of individual markers (AUC = 0.939, sensitivity = 0.876, specificity = 0.800) and that the combination of PIVKA-II and AFP was the most valuable panel for detecting HCC (AUC = 0.890, sensitivity = 0.901 specificity = 0.648). Choi et al. [27] found that the combination of AFP-L3 and PIVKA-II could increase sensitivity to 0.944. Although the combination of three or four markers was more effective than that of DNASE1L3 and AFP (AUC = 0.931, sensitivity = 0.886), its cost of examination also increased, reducing its potential use. Generally, DNASE1L3 showed better diagnostic effectiveness than AFP in differentiating HCC from non-HCC hepatic diseases. In addition, the combination of the two markers could significantly improve the diagnostic performance of HCC. The detection methods are simple, stable, and reliable, thus making these markers suitable for application in hospitals at all levels. This study has some limitations. First, the sample size is relatively small. Second, the differences in qualitative characteristics, such as ethnicity, smoking, drinking, tumor stage and tumor size, were not analyzed because of incomplete clinical data. Third, we regret the unavailability of liver samples from HCC patients to determine the local expression of DNASE1L3. Therefore, these factors could produce potential bias for our results. Previous studies have shown that serum DNASE1L3 levels could show different levels in the development of HCC and that monitoring serum DNASE1L3 levels in various HCC stages could provide reliable laboratory evidence for disease progression and treatment effects. Therefore, further investigations on serum DNASE1L3 are required to validate its great potential. They can help us to understand the pathogenic mechanisms of HCC, identify the diagnostic biomarkers for HCC, and design new therapeutic strategies to improve the effectiveness of HCC treatment.

CONCLUSION

In this study, the serum DNASE1L3 level significantly increased in HCC, thus showing that it has greater diagnostic effectiveness than AFP in differentiating HCC from non-HCC hepatic diseases. The combination of DANSE1L3 and AFP had higher sensitivity and AUC, thus significantly improving the diagnostic performance of HCC. Future studies are needed to highlight the underlying mechanisms and the predictive value of DNASE1L3 in the outcomes of HCC patients.

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Declaration of Interest:
The authors declare no competing interests.

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