Serum Biomarkers Study and the Establishment of Diagnostic Models for Hepatitis B Virus-Related HCC

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Abstract: China's HCC accounts for 90% of HBV related HCC. Early detection, diagnosis and treatment are the key to effective control of HCC. By measuring the levels of expression of AFP, DCP and GP73 in the serum of HBV-related HCC patients, the diagnostic value of single and combined detection of the above indicators in HBV-related HCC shall be discussed, and the mathematical model of differential diagnosis by SVM shall be established to provide reference for the diagnosis of HBV-related HCC. A total of 301 patients and healthy persons from March 2016 to January 2018 from Beijing Tongren Hospital affiliated to Capital Medical University have been selected. These lection includes 57 cases of HBV-related HCC, 61 cases of non- HBV-related HCC, 52 cases of HBV-related cirrhosis, 57 cases of chronic HBV, and 74 healthy persons in the same period. The levels of serum DCP, AFP and GP73 in each group were measured. Combined diagnosis of three indexes is better than single diagnosis, P<0.001. Using SVM mathematical diagnosis model, the specificity and sensitivity of diagnosing HBV-related HCC and healthy controls reached 98.7% and 97.6%, while the specificity and sensitivity of diagnosing HBV-related HCC and HBV-related cirrhosis reached 90.91% and 96.3%, respectively. Serum DCP, AFP and GP73 can be used independently as a useful reference for diagnosing HBV-related HCC patients. Combined detection of the three indicators can improve the sensitivity of HBV-related HCC diagnostic test. The SVM model can be used to diagnose and identify liver diseases at different stages.

Keywords: Primary hepatocellular carcinoma, des-γ-carboxy-prothrombin, alpha fetoprotein, golgi protein 73, diagnostic value: support vector machine.

1. INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors. The incidence and mortality of HCC are among the leading malignant tumors in the world [1-2], accounting for 18.8% of the death of malignant tumors in China [3], with a low 5-year survival rate of only 5-9% [4]. The hepatitis B virus (HBV) and hepatitis C virus (HCV) infection is the main etiological factors of HCC. According to statistics, China's HCC accounts for 90% of HBV-related HCC [5-6]. Early detection, early diagnosis and early treatment are the key to effective control of HCC. HCC diagnosis mainly depends on imaging and pathology results. Unfortunately, imaging and pathology testing are extremely costly, unsuitable for monitoring high-risk groups, and are largely dependent on the use of instruments and the doctor's experience. Therefore, it is necessary to discover a highly sensitive and specific tumor markers to identify HCC. The sensitivity of alpha-fetoprotein (AFP) is 40% ~ 65%. When HCC lesions are small, the false negative rate detected by AFP is high [7], and the diagnostic efficacy needs to be improved. DCP, also known as protein induced by vitamin K deficiency or antagonist - II (PIVKA - II), is an abnormal prothrombin with no coagulation activity synthesized by liver cancer, which is an important indicator to judge the progression and prognosis of liver cancer.

However, these markers often give false positive results and lack sufficient sensitivity and specificity [8]. Golgi protein 73 (GP73) is a specific membrane protein expressed by the liver bile duct epithelial cells. In HBV and HCV infection related HCC patients, there is a significant rise in serum concentration of GP73, and studies have shown that the efficiency of GP73 levels determination for the diagnosis of HCC and the GP73 high or low concentration values in benign and malignant liver disease are controversial, so GP73 value for the diagnosis of HCC is not superior to AFP [9].

In this study, the expression of AFP, GP73 and DCP in serum of patients with HBV-related HCC was measured, and the diagnostic value of single or combined detection of the above indicators in HBV-related HCC was explored, which could provide a reference for the screening and diagnosis of HBV-related HCC. No scholar has applied serum markers to study the system of HBV-related HCC and to establish a screening and diagnostic model. This study innovatively identifies specific diagnostic markers for

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screening HBV-related HCC patients. Meanwhile, the establishment of SMV model and its effect evaluation are analyzed, and a variety of clinical screening and diagnostic models are established. Combined with multiple serological markers, we established a model for screening and diagnosis of HBV related HCC.

2. OBJECTS AND METHODS

2.1. The Objects of Study

A total of 301 patients and healthy persons from March 2016 to January 2018 from Beijing Tongren Hospital affiliated to Capital Medical University were selected. The patients were divided into five groups according to their disease status: 57 patients in hepatitis B related HCC (HBVHCC), 61 patients in non-hepatitis B related HCC (NHBVHCC), 52 patients with hepatitis B liver cirrhosis (LC). 57 patients with hepatitis B (HBV) were enrolled in the control group, and 74 healthy controls (HC) were enrolled in the control group.

2.2. Inclusion and Exclusion Criteria

The criteria for HCC group are in accordance with the "Standards for Diagnosis and Treatment of HCC " of the Ministry of Health of the People's Republic of China, 2011 [10], HBV related HCC was diagnosed by clinical manifestations, pathological findings, imaging and other related examinations. Chronic HBV infection was found. No anti-tumor measures such as surgery, radiofrequency ablation, interventional therapy, radiotherapy and chemotherapy were performed. As for HBV- related LC group, the criteria are in accordance with the Guidelines for the Prevention and Treatment of HBV updated by the Society of Hepatology of Chinese Medical Association in 2015 [11]. The patients with LC were diagnosed by clinical manifestations, biochemical, histopathological examination or liver ultrasonography. Diagnostic criteria for HBV were no evidence of LC found in liver histopathological examination or on clinical, laboratory and imaging examination.The following shows the criteria for HC group: age over 18 years with no apparent disease found on physical and laboratory examination. Those who had taken vitamin K preparations or vitamin K antagonists such as dicoumarin and warfarin; those with primary malignant tumors of any other organ or metastatic HCC, with other hepatitis virus infections, alcoholic liver disease, non-alcoholic fatty liver disease, autoimmune liver disease, drug-induced liver damage and hereditary metabolic diseases; with liver damage caused by other causes such as schistosomiasis hepatica; pregnant or lactating women were excluded from the study.

2.3. Sample Collection

After signing of the informed consent form by the patients, blood sample were extracted from patients' cubital vein using a disposable blood sampler in the morning under fasting conditions and put it into the vacuum blood collection vessel with separating glue.

After 30 minutes at room temperature, the serum was separated by 3500 r/min centrifugation for 15 minutes for the detection of tumor markers. The supernatant was transferred into 2ml Eppendorf tube; placed in the vacuum blood collection vessel with separating glue and store at -80 C.

2.4. Research Methods

Serological markers of HCC i.e, DCP was detected by chemiluminescent enzyme immunoassay, AFP was detected by electrochemiluminescent assay as reagent of Roche reagent; GP73 was detected by UPT as reagent of Beijing Rejing Biotechnology Co., Ltd.

2.5. Statistical Methods

SPSS22.0 software was used for statistical analysis. Measuring data conforming to normal distribution are expressed by mean±standard deviation (X ±S). Measuring data of non-normal distribution are expressed by median (M) and quartile spacing (Q25, Q75), counting data is expressed in percentage (%)。Normality and homogeneity of variance tests were performed for the comparison of mean values of multiple samples. Those who met the above conditions were compared by variance analysis. On the contrary, Kruskal-Wallis H test was used for comparison of multiple independent samples. Mann-Whitney U test was used for comparison between groups. Receiver operator characteristic curve (ROC curve) was drawn to determine the best critical value (Cut-off value). The area under the Curve (AUC) was compared by Z test. The test level was alpha = 0.05, $P < 0.05$.

3. RESULTS

3.1. Comparison of Basic Data between HBV Related HCC Group and other Control Groups

The age and sex of HBV-related HCC group, Non-HBV-related HCC group, HBV -related LC group, HBV group and HC were statistically analyzed. The results,

Table 1 Comparison of Basic Data of Five Groups of Subjects

group	number	Age (year)	Sex (male / female)
HBV-related HCC	57	54.22±10.89	30/27
NHBV-related HCC	61	50.26±12.79	32/29
HBV LC	52	47.83±11.89	27/25
HBV	57	51.57±12.49	31/26
HC	74	45.22±10.09	39/35
P value		>0.05	>0.05

as depicted in Table **1**, showed that there was no significant difference in the basic data within the five groups (P > 0.05).

3.2. Serum Levels of AFP, GP73 and DCP

In order to conveniently observe and analyze the values of the three serum tumor markers, we took logarithm for each serum level. DCP and AFP did not satisfy the normal distribution by taking the Shapiro-Wilk test. Therefore, Kruskal-Wallis H test was used for the contents of DCP, GP73 and AFP (H value was 104.27, 79.45, 45.681, *P* value was $P < 0.001$, $P < 0.001$), respectively. The Mann-Whitney U test was used between the two groups, while the GP73 test data met normal distribution, and t test was used among the groups. Results are shown in Table **2**.

3.2.1. Serum DCP Level

HBV group and HBVHCC group, NHBVHCC group had significant differences in DCP content (U value was 290, 210.5, 269, *P* value was $P = 0.888$, $P = 0.008$, $P = 0.02$ respectively); DCP content in LC, HBVHCC and NHBVHCC groups had no significant difference (U value was 255, 296, *P* value was $P = 0.399$, $P = 0.417$) (Results are shown in Figure **1A**).

3.2.2. Serum GP73 Level

There were significant differences between HC group and HBV LC, HBVHCC, NHBVHCC group in GP73 content (t value was - 14.579, - 12.007, - 6.871, *P* value was $P < 0.001$, $P < 0.001$), HBV group and LC, HBVHCC group, NHBVHCC group in GP73 content (t value was - 6.847, - 6.524, - 3.976, $P < 0.001$, respectively). And there was significant difference in GP73 content between HBV LC group and NHBVHCC group (t value was 0.776, 3.227, *P* value was 0.442, 0.002,respectively) (Results are shown in Figure **1B**).

3.2.3. Serum AFP Level

There was significant difference in AFP content between HC group and HBV LC, HBVHCC group and NHBVHCC group (value was 189.5, 345, 644, *P* value was respectively ($P < 0.001$, $P < 0.001$,$P < 0.001$); But there was no significant difference in AFP content between HBV group and LC, HBVHCC group (U value was 163, 177, 294, *P* value was $P = 0.007$, respectively). (Results are shown in Figure **1C**).

3.3. Diagnostic Value of Combined Detection of Serum DCP, GP73 and AFP in Hbvrelated Hepatocellular Carcinoma

3.3.1. HBV-Related HCC and HC (Results are Shown in Figure 2A)

The AUC and 95% confidence interval of each index were obtained. The sensitivity, specificity, positive predictive value and negative predictive value were obtained (Results are shown in Table **3**). The results are displayed as follows: $AUC_{DCP} = 0.848$, 95%

Table 2: Detection Results of DCP, AFP and GP73 in Serum (M Q25, Q75)

Group	number	DCP		GP73		AFP	
		Median	Q25, Q75	Median	Q25, Q75	Median	Q25, Q75
HBV related HCC	27	20.330	7.530,91.000	172.200	106.200,252.000	3.740	0.830,140.710
NHBV related HCC	31	14.420	5.590,40.620	101.300	73.100,185.000	1.460	0.510,2.250
HBV Liver cirrhosis	22	6.150	3.573,177.048	162.200	147.225,237.050	2.080	1.303,9.448
HBV	27	6.810	4.510,10.730	61.310	46.200,82.390	0.420	0.100,4.170
Healthy control	74	2.115	1.183,3.430	50.775	41.583,63.655	0.690	0.400,1.098

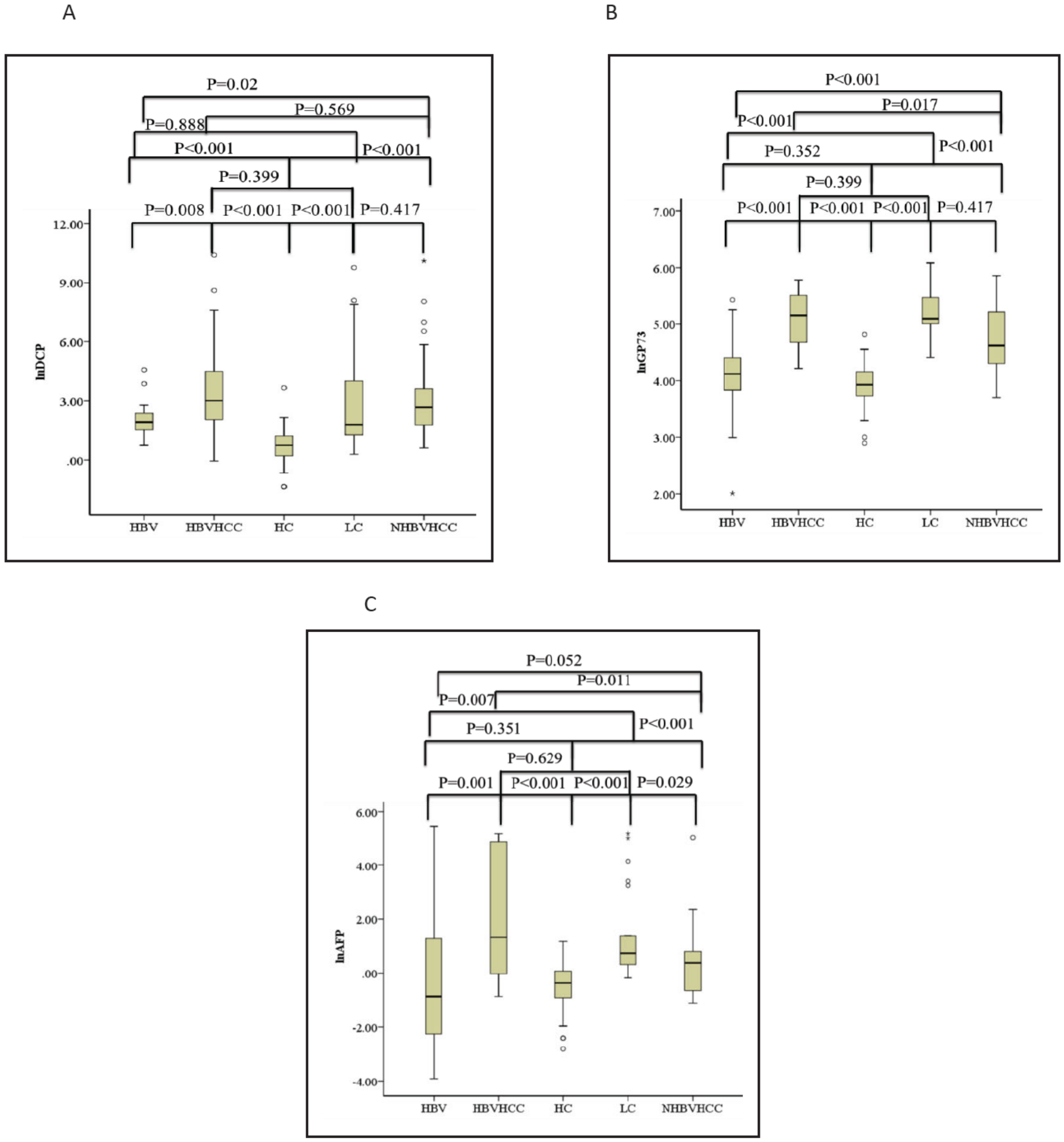

Figure 1: Serum levels of AFP, GP73 and DCP.

CI (0.741-0.955), the optimum critical value is 6.705ng/ml whilst the sensitivity, specificity, positive predictive value and negative predictive value are 77.8%, 97.3%, 91.3%, 92.3%, AUC_{GP73} = 0.984, 95% CI (0.965-1.0), and the optimum critical value is 84.31ng/ml whilst sensitivity, specificity and positive predictive value and negative predictive values were 92.6%, 95.9%, 89.3% and 97.3% respectively; AUC_{AFP} = 0.827, 95% CI (0.728-0.927), and the optimal critical value was 1.497ng/ml whilst the sensitivity, specificity, positive predictive value and negative predictive value were 66.7%, 89.2%, 69.2% and 88%, respectively. Logistic regression analysis was used to obtain the combined predictive factors, and ROC curves were drawn for the combined diagnosis of the three indicators. The results showed that $AUC_{DCP+GP73+AFP}$= 0.997, 95% CI (0.4930-1.0), sensitivity, specificity, positive predictive value and negative predictive value were 100%, 98.6%, 96.4% and 100%, respectively. Combined diagnosis of three indexes is better than single diagnosis, *P*<0.001.

3.3.2. Diagnostic Value of 2. DCP, GP73 and AFP in High-Risk Group

3.3.2.1. HBV-Related HCC and HBV-Related LC (Results are Shown in Figure **2B**)

The AUC and 95% confidence interval of each index were obtained, and the sensitivity, specificity,

Table 3: Comparison of Diagnostic Value of DCP, GP73 and AFP in Each Group

Index	AUC(95%CI)	critical value	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
HBVHCC*VS*HC DCP	0.848(0.741-0.955)	6.705	77.8	97.3	91.3	92.3
GP73	0.984(0.965-1.000)	84.310	92.6	95.9	89.3	97.3
AFP	0.827(0.728-0.927)	1.494	66.7	89.2	69.2	88
Combination of three projects	0.997(0.990-1.000)	1.2874	100	98.6	96.4	100
HBVHCC*VS*LC						
DCP	0.571(0.403-0.739)	11.219	70.4	59.1	67.9	61.9
GP73	0.459(0.294-0.623)	168.208	51.9	59.1	60.9	50
AFP	0.540(0.374-0.707)	4.559	48.1	77.3	72.2	54.8
Combination of three projects	0.650(0.493-0.807)	1.710	66.7	68.2	72	62.5
HBVHCC*VS*HBV						
DCP	0.711(0.556-0.867)	13.952	66.7	88.9	58.1	72.7
GP73	0.919(0.846-0.992)	84.792	92.6	81.5	83.3	91.7
AFP	0.757(0.625-0.890)	0.575	88.9	59.3	68.6	84.2
Combination of three projects	0.919(0.846-0.992)	1.344	100	74.1	79.4	100
HBVHCC*VS*HBV+LC						
DCP	0.648(0.502-0.794)	13.951	66.7	75.5	60	80.4
GP73	0.712(0.598-0.827)	84.792	92.6	46.9	49	92
AFP	0.660(0.535-0.785)	0.383	100	26.5	42.9	100
Combination of three projects	0.728(0.615-0.814)	1.230	100	38.8	47.4	100

positive predictive value and negative predictive value were obtained (Results are shown in Table **3**). The results are as follows: AUC_{DCP} = 0.57, 95% CI (0.403-0.739), the best critical value is 11.219ng/ml, sensitivity, specificity, positive predictive value and negative predictive value are 70.4%, 59.1%, 67.9%, 61.9%, AUC_{GP73} = 0.459, 95% CI (0.294-0.623), the best critical value is 168.208 ng/ml, sensitivity, specificity, positive predictive value and negative predictive value are respectively. The negative predictive values were 51.9%, 59.1%, 60.9% and 50% respectively (Results are shown in Table **3**); AUC_{AFP} = 0.540, 95% CI (0.374-0.707), and the optimal critical values were 4.559 ng/ml sensitivity, specificity, positive predictive value and negative predictive value: 48.1%, 77.3%, 72.2%, 54.8%, respectively. Logistic regression analysis was used to obtain the combined predictive factors, and ROC curves were drawn for the combined diagnosis of the three indicators. The results showed that $AUC_{DCP+GP73+AFP}$=0.650, 95% CI (0.493-0.807), sensitivity, specificity, positive predictive value and negative predictive value were 66.7%, 68.2%, 72% and 62.5%, respectively. Combined diagnosis of three indexes is better than single diagnosis, *P*<0.001.

3.3.2.2. HBV-Related HCC and HBV (Results are Shown in Figure **2C**)

The AUC and 95% confidence interval of each index were obtained, and the sensitivity, specificity, positive predictive value and negative predictive value were obtained (Results are shown in Table **3**). The results were as follows: AUC_{DCP} = 0.711, 95% CI (0.556-0.867), the best critical value was 13.952ng/ml, sensitivity, specificity, positive predictive value and negative predictive value were 66.7%, 88.9%, 58.1%, 72.7%. AUC_{GP73} = 0.919, 95% CI (0.846-0.992), the best critical value was 84.792ng/ml, sensitivity, specificity and positive predictive value were 84.792ng/ml. The negative predictive values were 92.6%, 81.5%, 83.3%, 91.7%, AUC_{AFP} = 0.757, 95% CI (0.625-0.890), the optimal critical value was 0.575 ng/ml, sensitivity, specificity, positive predictive value

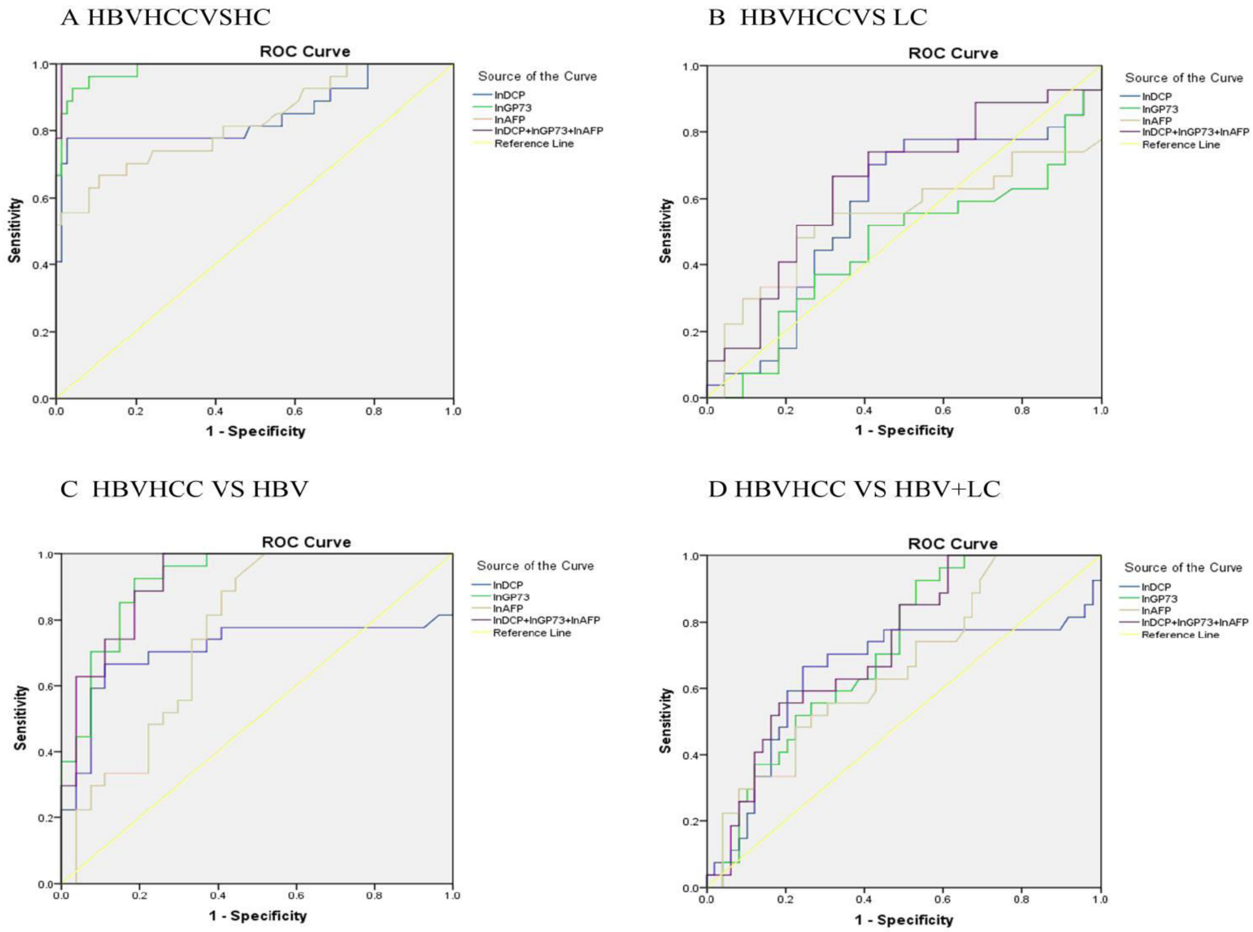

Figure 2: The curve (AUC) of serum DCP, GP73 and AFP ROC curves.

and negative predictive value were 88.9%, 59.3%, 68.6%, 84.2%, respectively. Logistic regression analysis was used to obtain the combined predictive factors, and ROC curves were drawn for the combined diagnosis of the three indicators. The results showed that $AUC_{DCP+GP73+AFP}$=0.919, 95% C I (0.493-0.807), sensitivity, specificity, positive predictive value and negative predictive value were 100%, 74.1%, 79.4% and 100%, respectively. Combined diagnosis of three indexes is better than single diagnosis, *P*<0.001.

<u>3.3.2.3. HBV-Related HCC and HBV+ HBV-Related LC (Results are Shown in Figure **2D**)</u>

The AUC and 95% confidence interval of each index were obtained, and the sensitivity, specificity, positive predictive value and negative predictive value were obtained (Results are shown in Table **3**). The results were as follows: AUC_{DCP} = 0.648, 95% CI (0.502-0.794), the best critical value was 13.951ng/ml sensitivity, specificity, positive predictive value and negative predictive value were 66.7%, 75.5%, 60%, 80.4%, AUC_{GP73} = 0.712, 95% CI (0.598-0.827), the best critical value was 84.792ng/ml, sensitivity, specificity, positive predictive value, negative predictive value, respectively. Sex predictive values were 92.6%, 46.9%, 49%, 92%, AUC_{AFP} = 0.660, 95% CI (0.535-0.785), the best critical value was 0.383 ng/ml, sensitivity, specificity, positive predictive value and negative predictive value were 100%, 26.5%, 42.9%, 100%, respectively. Logistic regression analysis was used to obtain the combined predictive factors and draw ROC curve for the combined diagnosis of the three indicators. $AUC_{DCP + GP73 + AFP}$ = 0.728, 95% C I (0.615-0.814), sensitivity, specificity, positive predictive value and negative predictive value were 100%, 38.8%, 47.4% and 100%, respectively. Combined diagnosis of three indexes is better than single diagnosis, *P*<0.001.

3.4. SVM Model Establishment and Effect Evaluation

SVM, a learning method based on statistical learning theory and structural risk minimization principle, is a multivariate statistical analysis method, which is widely used in multivariate analysis and pattern recognition [12-14]. The basic principle is to seek an appropriate hyperplane for solving the classification issue by selecting the corresponding kernel function in mapping the original data to a higher dimensional space [15]. Here we choose the radial basis kernel function in the model [16]:

$$K(x_i, x_j) = \exp\left(-\frac{\| x_i - x_j \|^2}{\gamma^2}\right)$$

where γ is the nuclear parameter. In addition, penalty parameter C is used when searching for the optimal hyperplane [16]. Both parameters, determining the efficiency and generalization ability of the model, should be optimized by employing the algorithm combining mesh grid search with five-fold cross validationin this paper.It is prudent to select training set to build SVM model because the terrible diagnosis and prediction results could be reached by the poor model. Generally, there are two methods, Random-Select (R-S) [17] and Kennard-Stone (K-S) [18] algorithm, to divide the dataset into the training set and testing set. Due to the randomization of R-S and unrepresentativeness in the selected data, the unavoidable fluctuation of the prediction model will deviate from the true results. In contrast, the prediction model is stable with the representative selected data in K-S algorithm that is based on the Euclidean distances within the dataset. In this work, we employed K-S algorithm to select two thirds of the original data as the training set, the remaining data was test data.

From the results of Table **4**, It was shown that SVM has a good diagnostic effect in HBV-related HCC group and HBV LC, HBV, HC group. When diagnosing HBV-related HCC and HC, the specificity and sensitivity of SVM reached 98.7% and 97.6%, and when diagnosing HBV-related HCC and HBV-related LC, the specificity and sensitivity reached 90.91% and 96.3%.

4. DISCUSSION

HBV infection is an important reason for the development of HCC in China.

The 5-year survival rate of HCC is 40%, which can be increased to 60-70% by early hepatectomy [19]. Pathological biopsy is the gold standard for the diagnosis of HCC, but because it is an invasive examination, the difficulty and risk of puncture exist. Imaging examination has limited ability to detect small HCC. Serological biomarkers are non-invasive, simple and objective which are mostly used in early screening and diagnosis of HCC as well as in monitoring and management of HCC.

Serum AFP detection and abdominal ultrasound examination every six months have become a common screening method for high-risk groups of HCC [20]. However, some patients with confirmed HCC have no significant increase in AFP, while some patients with non-HCC have elevated AFP. Because of the low sensitivity and specificity of AFP in screening and diagnosis of HCC, in recent years, the European Society for Liver Research and the American Society for Liver Disease Research no longer use AFP as a screening and diagnostic criterion for HCC in their updated guidelines for diagnosis and treatment [21-22]. In this study, the sensitivity of AFP in the diagnosis of HCC is only 66.7%, which means that nearly 40% of HCC patients may be missed, so we still need to be alert to the risk of HCC in high-risk population with negative AFP. Serum AFP can also be increased in patients with LC. There is no significant difference between HBV-related HCC group and HBV-related LC group, which may be related to the small number of samples and the degree of disease. HBV-related HCC is common in China. Whether the serum level of AFP is different in HCC caused by different etiologies needs to be further explored.

The diagnostic value of DCP for HCC has been recognized internationally. The detection of serum DCP in Japan has been included in the project of screening management for high-risk groups of HCC [23]. This study found that the serum DCP level of HBV-related HCC patients was higher than that of the HC group, and the difference was significant. Therefore, serum DCP has high sensitivity and specificity in the diagnosis

Table 4: SVM Model Establishment and Effect Evaluation

group	C	γ	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
HC-HBVHCC	0.6598	16	98.7	97.6	96.5	98.6
LC-HBVHCC	13.9288	13.9288	96.30	90.91	92.86	95.24
HBV-HBVHCC	445.7219	0.3299	96.30	88.89	89.66	96
HBV+LC-HBVHCC	1.1487	1.3195	29.63	95.92	80	71.21

of HBV-related HCC, which is helpful for the screening of HBV-related HCC. At the same time, the negative predictive value of serum DCP is high, which has a certain clinical value for the elimination of HCC.

GP73 is mainly expressed in bile duct epithelial cells in normal liver tissues. The serum concentration of GP73 is significantly increased in patients with HCC associated with HBV and HCV infection. GP73 is recommended as a diagnostic index for HCC because of its abnormal elevation in serum of patients with HCC. This study found that the serum GP73 level in patients with HBV-related HCC was significantly higher than that in the control group. When the optimal critical value was 84.310 ng/ml, the sensitivity, specificity, accuracy, positive predictive value and negative predictive value were 92.6%, 95.9%, 89.3% and 97.3%, respectively. However, there was no significant difference between HBV-related LC group and HBV-related HCC patients, suggesting that the detection of serum GP73 is difficult to differentiate hepatocirrhosis from HCC, and serum GP73 can be used as an effective serological diagnostic index reflecting the severity of hepatic fibrosis to a certain extent.

In this study, compared with HC group and HBV group, the AUC of HBV-related HCC was between 0.7 and 0.9, which indicated that the three indexes could be used to differentiate HBV-related HCC from HC and HBV with a certain accuracy. However, it is noteworthy that the three indicators are ineffective in differentiating HBV-related HCC from LC, but considering that HBV-related HCC may be accompanied by LC in clinic, the sample size of this study is too small, and future prospective studies with a larger sample size can further reduce the bias caused by other factors. However, the single serological index still has the defect of low sensitivity, which requires a combination of multiple serological indicators to improve the diagnostic rate. In this study, it showed that combined detection of three indicators greatly improved the sensitivity of diagnosis of HBV-related HCC and could improve the diagnostic accuracy of HBV-related HCC. The higher detection rate of HBV-related HCC has reduced missed diagnosis rate, which is helpful for early diagnosis of HCC. Moreover, the negative predictive value of combined detection is as high as 100%, which is of great reference value to the exclusion diagnosis of HCC.

However, it should not be neglected that while the sensitivity of the combined detection of the three indicators increases, the specificity decreases, hence the misdiagnosis rate may increase. It is still necessary to make a comprehensive judgment based on the general health condition of the patients, clinical signs and symptoms, laboratory results, pathological or imaging data in order to improve the detection rate and at the same time minimize misdiagnosis rate.

The innovation of this study uses SVM model to diagnose HBV-related HCC and HBV-related LC, HBV and HC. The specificity and sensitivity of SVM are 98.7% and 97.6% in diagnosing HBV-related HCC and HC, and 90.91% and 96.3% in diagnosing HBV-related HCC and LC, respectively. The diagnostic effect of these two SVMs is better than that of tumor markers' diagnostic effect. Diagnostic effect. Therefore, the SVM model can be used to diagnose and identify liver diseases at different stages.

The inadequacy of this study lies in the insufficiency of the cases included, the different conditions of the selected cases, and the introduction of selective bias. Therefore, the conclusions obtained through the analysis need to be confirmed by large sample, multi-center and long-term follow-up studies. Our diagnostic model provides ideas and methods to improve the early diagnosis of HCC, but it is obviously not ideal to rely solely on the above model for early diagnosis of HCC. Combined with the corresponding imaging indicators, the detection rate of early HCC may be further improved.

5. CONCLUSION

Serum DCP, AFP and GP73 can be used independently as a useful reference for diagnosing HBV-related liver cancer patients. Moreover, combined detection of the three indicators can improve the sensitivity of HBV-related liver cancer diagnostic test. In addition, the SVM model can be used to diagnose and identify liver diseases at different stages.

DECLARATIONS

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Availability of Data and Materials

Please contact the corresponding author for all data on reasonable request.

Authors' Contributions

Xiangyi Liu and Jing Bai designed the experiments. Jing Bai , Haishen Liu and Hongfei Wan collected clinical samples. Jing Bai and Haishen Liu performed experiments and analyzed data. Xiangyi Liu and Jing Bai interpreted the results. The manuscript was drafted by Jing Bai, and edited by Xiangyi Liu. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

This study was approved by the Institutional Review Board of Tongren Hospital (approval number 2016-0907). All subjects gave written informed consent.

Patient Consent for Publication

Not applicable.

Competing Interests

The funding organization played no role in the study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

LIST OF ABBREVIATIONS

AFP	=	alpha fetoprotein
AUC	=	area under the Curve
DCP	=	des-γ-carboxy-prothrombin
GP73	=	golgi protein 73
HBV	=	hepatitis B virus
HC	=	healthy controls
HCC	=	hepatocellular carcinoma
LC	=	liver cirrhosis
ROC curve	=	receiver operator characteristic curve
SVM	=	support vector machine

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