RESEARCH ARTICLE

Combined detection of AFP-L3, GP73 and TIP30 enhances diagnostic accuracy for HBV-related cirrhosis and hepatocellular carcinoma

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Abstract

Objective: To investigate the value of combined tests of serum Golgi protein-73, alpha-fetoprotein-L3 and Tat-interacting protein-30 in the diagnosis of hepatitis B virus related cirrhosis and hepatocellular carcinoma.

Methods: The cross-sectional study was conducted at Yuebei People's Hospital, Guangdong, China, from January to October 2017, and comprised hepatitis B patients and healthy controls. Serum Golgi protein-73, alpha-fetoprotein-L3 and Tat-interacting protein-30 levels in both groups were detected by enzyme-linked immunosorbent assay (ELISA). Alpha-fetoprotein-L3 was separated and quantified by electrochemiluminescence immunoassays and the percentage of alpha-fetoprotein-L3 to alpha-fetoprotein was calculated.

Results: Of the 721 subjects, 525(%) were patients and 196(%) were healthy controls. Among the patients, 271(%) had chronic hepatitis B, 161(%) had liver cirrhosis and 93(%) had hepatocellular carcinoma. Serum Golgi protein-73, alpha-fetoprotein-L3 and Tat-interacting protein-30 levels were significantly different in the hepatocellular carcinoma patients compared to controls, and those with chronic hepatitis and liver cirrhosis ($p<0.01$ each). The sensitivity and specificity of the combined detection of the three serum levels for diagnosing cirrhosis were 78.26% and 86.72%. The corresponding values for diagnosing hepatocellular carcinoma were 86.02% and 92.51%.

Conclusions: Combined detection of Golgi protein-73, alpha-fetoprotein-L3 and Tat-interacting protein was found to have the potential to improve diagnostic accuracy.

Keywords: Hepatocellular carcinoma, Cirrhosis, Chronic hepatitis B, Tumour marker.

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Introduction

[1]Hepatocellular carcinoma (HCC), with the fifth highest global incidence, the second highest morbidity and third highest mortality rate in China, is one of the leading causes of cancer-related mortality worldwide due to its poor prognosis. The main causes of HCC are viral hepatitis, aflatoxin pollution, smoking and alcohol. Also, infection with hepatitis B virus (HBV) may lead to chronic liver inflammation with the risk of developing liver cirrhosis (LC) and HCC. HBV infection is closely associated with the occurrence of HCC, with a correlation of 80%.[1,2] If personal diet and living habits are neglected, and there is no timely antiviral treatment or hepatoprotective therapy, the majority of the patients move from chronic hepatitis B (CHB) to liver fibrosis, cirrhosis and, ultimately, liver cancer.[3] As the most important tumour marker for the diagnosis of HCC, alpha-fetoprotein (AFP) has been used for decades with a sensitivity of 40-65% and a specificity of 76-96%.[4]

In recent years, Golgi protein-73 (GP73) and AFP-L3 have been used as new tumour markers for the early diagnosis of HCC. As a transmembrane protein in the Golgi body, GP73 is mainly expressed in bile duct epithelial cells, whereas it is rarely or seldom expressed in hepatocytes. The serum GP73 levels increase in patients with viral hepatitis B or C, or other chronic liver diseases, like alcoholic or fatty liver disease, with a sensitivity of 69% and a specificity of 75%, which are superior to those of AFP.[5,6] According to the different affinity between AFP

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and phytolectin, AFP is divided into AFP-L1, AFP-L2, and AFP-L3; AFP-L3 has been proven beneficial in early diagnosis, with a sensitivity of 72.3% and a specificity of 97.2%, which indicates its value in the evaluation of the aggressiveness and prognosis of HCC.[7,8] Tat interacting protein 30 (TIP30) or human immunodeficiency virus-1 (HIV-1) Tat interactive protein-2 (HTATIP2), considered to be the expression products of tumour suppressor genes, were first found during a study of the transcription of HIV which showed that lower TIP30 expression was associated with the occurrence and transfer of HCC.[9] To date, there have been reports on the significance of GP73 and AFP-L3 single and combined detection in HCC diagnosis, but, to the best of our knowledge, there have been no reports on serum TIP30 in HCC diagnosis and the disease process of CHB, LC and HCC. The current study was planned to fill this gap in literature.

Patients and Methods

The retrospective cohort study was conducted at Yuebei People's Hospital, Guangdong, China, from January to October 2017, and comprised HBV patients and healthy controls.

After approval was obtained from the institutional ethics review board, PASS 15.0 was used to calculate the sample size in line with literature.[10] Those included were patients aged >20 years with HBV infection suffering from CHB, LC or HCC. Those excluded were patients with concomitant infection by other types of hepatitis virus, concomitant liver injury caused by alcohol and drugs, concomitant liver metastasis from other tumours, previously diagnosed HCC, history of liver transplantation or liver resection for a reason other than HCC, and any cancer other than HCC. The control group had healthy individuals aged >20 years with normal blood cell count, liver function and blood coagulation function. Those excluded from the control group were individuals with viral hepatitis, fatty liver, alcoholic liver, drug hepatitis and other liver injury or diseases. Also excluded were those with surgical history within the preceding 6 months, chronic disease history, unhealthy lifestyles like insufficient sleep, alcohol abuse, smoking or drug use), abnormal liver function and HBV deoxyribonucleic acid (DNA) detection, or any physical discomfort. CHB was confirmed by time-resolved fluoroimmunoassay or HBV DNA test.

LC was defined by histological information or clinical criteria. When the histological information was not available, cirrhosis was defined as follows: i) Liver stiffness measurement (LSM) =17.5 kpa, detected by FibroScan, which is a diagnostic device based on transient elastography (TE); aspartate aminotransferase(AST)-to-platelet (PLT) ratio index (APRI) >2; Fibrosis index based on the 4 factor (FIB-4)>3.25; ultrasonographic findings suggestive of cirrhosis, including a blunted, nodular liver edge accompanied by splenomegaly (>12cm); oesophageal or gastric varices; or overt complications of LC, such as ascites, variceal bleeding and hepatic encephalopathy.

The diagnosis of HCC was obtained either histologically or non-invasively, and was based on the diagnostic criteria of primary hepatic carcinoma published by the China Health and Family Planning Bureau in 2017. Combined with the high-risk factors, imaging characteristics and molecular markers of liver cancer, the clinical diagnosis of HCC was made based on the steps of the road map. Briefly, HCC was diagnosed when the typical characteristics of HCC (hypervascular in the arterial phase with washout in the portal venous or delayed phases) were observed using one of the following imaging techniques: Computed tomography or enhanced magnetic resonance imaging. Under suboptimal conditions, HCC diagnosis was confirmed by using two imaging techniques or liver biopsy. The Edmondson-Steiner staging system[11] was used for HCC staging. Small HCC was defined as a single cancer nodule of maximum diameter <3 cm, or a number of nodules <2 and a maximum diameter totalling <3 cm.

Technical Information

Fasting venous blood 5ml was collected from all patients. It was centrifuged at 3,500 rpm, and the plasma samples (>2ml) were frozen at -80^{O}C until analysis. Serum GP73 and TIP30 levels were detected by enzyme-linked immunosorbent assay (ELISA) (CUSABIO Inc., Maryland, USA). AFP-L3 was separated by microspin column (Hotgen Inc., Beijing, China). The levels of AFP-L3 in the separated eluent and total AFP were quantified by electrochemiluminescence immunoassays (Roche Inc., Tokyo, Japan), and the percentage of AFP-L3 to AFP was calculated. For patients with HBV-related HCC, samples were collected at the time of diagnosis, prior to commencing treatment. For HBV-related cirrhotic patients without HCC, the samples were obtained at the

time of diagnosis of cirrhosis. For CHB patients without cirrhosis or HCC, the samples were obtained at the time of diagnosis of CHB. The absence of HCC was confirmed at 1 year from the time of tumour marker measurement. SPSS 20 was used for statistical analysis. The data including median and interquartile range (IQR) was abnormally distributed, thus Kruskal-Wallis test of variance analysis was performed in multiple groups, and the Bonferroni test was adopted for comparisons between two groups. Count data was analysed using Chi-square test. A binary logistic regression analysis forecasting model was used for combined detection; the introduced standard was $p<0.05$ and the excluded standard was $p>0.1$. For the new variables of combined prediction probability, receiver operating characteristic (ROC) curves were produced for multi-index combined detection. The sensitivity, specificity and area under the ROC (AUROC) curve were used for the evaluation of sensitivity, specificity and accuracy of combined detection in the assisted diagnostic of HBV-related cirrhosis and HCC. $P<0.05$ was considered to indicate statistical significance.

Results

Of the 721 subjects, 525(%) were patients and 196(%) were healthy controls. Among the patients, 271(%) had CHB, 161(%) had LC and 93(%) had HCC. Baseline demographic and clinical characteristics of all the subjects were noted (Table 1)

Differences in tumour marker median levels in each group were compared (Table 2; Figure 1). The positive detection rate of GP73, AFP-L3 and TIP30 in LC patients was significantly higher compared to those with CHB and controls ($p<0.01$) (Table 3). The diagnostic efficacy of single and combined tests was evaluated by ROC curve (Figure 2; Table 4). The single TIP30 test exhibited the best diagnostic efficacy, and the diagnostic efficacy of the combined test was superior to that of any single test (Table 5).

Table-1: Baseline characteristics of the study population.

Variables	NC	CHB	LC	HCC	P-value
Total number (n)	196	271	161	93	
Male sex (%)	120 (61.22%)	172 (63.47%)	102 (63.35%)	58 (62.36%)	0.895
Age (years)	36.8 (32.0-40.2)	44.6 (35.6-53.2)	48.2 (38.2-55.5)	53.7 (39.5-65.7)	<0.001
Platelet count (109/l)	226.0 (112-320)	193.5 (95-266)	118.6 (76-145)	120.8 (80-142)	0.656
ALT (IU/l)	28.2 (18.9-35.3)	55.2 (20.2-89.3)	98.2 (51.3-150.3)	152.5 (83.1-245.5)	0.033
AST (IU/l)	22.0 (18.0-33.5)	23.5 (20.0-32.0)	33.0 (25.0-54.0)	45.0 (39.0-62.0)	0.013
Albumin (g/l)	44.2 (38.5-45.3)	39.8 (35.0-40.9)	37.5 (34.2-39.8)	35.6 (33.0-38.2)	0.016
Prealbumin (mg/l)	258.0 (209.8-306.2)	186.0 (146.5-225.5)	127.0 (90.8-163.2)	110.0 (79.4-140.6)	<0.001
Total bilirubin (μmol/l)	28.2 (25.0-31.4)	29.8 (26.2-33.4)	58.8 (51.3-66.3)	84.2 (74.3-94.1)	<0.001
LC	LSM (Kpa)	24.11 (18.38-29.84)			
	APRI	2.25 (1.45-3.05)			
	FIB-4	3.98 (3.37-4.59)			
	Child-pugh class A/B/C,n (%)	74 (45.96%)/56 (34.78%)/31 (19.25%)			
HCC	Small HCC n (%)	23 (24.73%)			
	Edmondson-Steiner class I-II/III-IV, n (%)	35 (37.63%)/58 (62.37%)			

Data are expressed as the number (percentage) and median (interquartile range). NC, normal control group; CHB, chronic hepatitis B group; LC, liver cirrhosis group; HCC, hepatocellular carcinoma group; ALT, alanine transaminase; AST, aspartate aminotransferase; LSM, liver stiffness measurement; APRI, aspartate aminotransferase-to-platelet ratio index; FIB-4, fibrosis index based on the 4 factor.

Table-2: Comparison of serum GP73, AFP-L3% and TIP30 test results in each group.

Groups	Number (N)	GP73 (IU/ml)	AFP-L3 (%)	TIP30 (ng/ml)
NC	196	0.092 (0.075-0.109)	9.874 (8.451-11.297)	11.997 (10.695-13.299)
CHB	271	0.908 (0.725-1.102)	9.403 (6.336-12.440)	11.027 (8.615-13.439)
LC	161	1.087 (0.857-1.317)	13.176 (11.154-15.198)	7.634 (5.373-9.895)
HCC	93	1.319 (1.049-1.589)	15.721 (13.604-17.838)	4.946 (3.580-6.312)
F value		291.385	83.143	131.163
P-value		<0.01	<0.01	<0.01

Data are expressed as the median value (interquartile range); *: Comparison between HCC and the other groups, P<0.001. CHB, chronic hepatitis B; LC, liver cirrhosis; HCC, hepatocellular carcinoma; GP73, Golgi Protein-73; AFP-L3, alpha-fetoprotein-L3' TIP30, Tat-interacting protein-30.

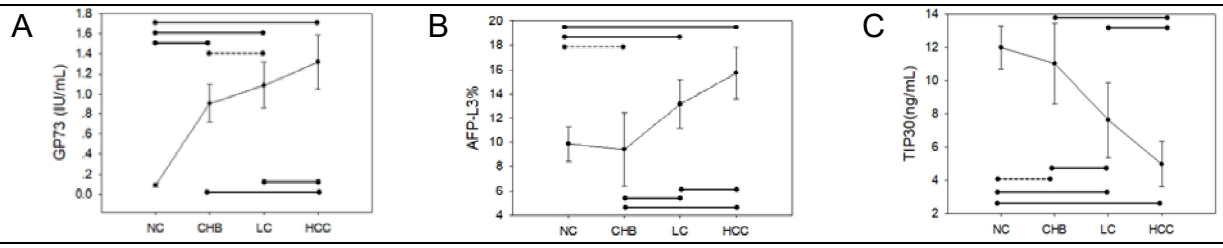

Figure-1: Comparison of serum ; Golgi Protein-73 (GP73); alpha-fetoprotein-L3 (AFP-L3) and Tat-interacting protein-30 (TIP30) values in patients of each group. The values of GP73 (A), AFP-L3% (B) and TIP30 (C) are shown as scatter charts with error bars. The solid line represents P<0.01, the dotted line represents P>0.05.

Table-3: Comparison among the positive ratio of serum GP73, AFP-L3 and TIP30 in diagnosis of HBV-related cirrhosis.

Groups	Cases (N)	GP73	AFP-L3 (%)	TIP30
LC	161	63.98 (103/161)*	60.25 (97/161)*	74.53 (120/161)*
CHB	271	35.79 (97/271)	31.73 (86/271)	29.52 (80/271)
NC	196	10.20 (20/196)	7.65 (15/196)	5.10 (10/196)
?2		52.514	45.506	72.367
P-value		0.000	0.000	0.000

Data are expressed as positive percentage (n/n). LC, liver cirrhosis group; CHB, chronic hepatitis B group; NC, normal control group; GP73, Golgi Protein-73; AFP-L3, alpha-fetoprotein-L3' TIP30, Tat-interacting protein-30

Table-4: Comparison of AUROCs of HBV-related cirrhosis diagnosed differently by single and combined detection of GP73, AFP-L3 and TIP30.

Tumor markers	AUROC	Std.ea	S.Ab	95% CI	
				Upper limit	Lower limit
GP73	0.846	0.031	0.000	0.785	0.908
AFP-L3	0.864	0.030	0.000	0.806	0.922
TIP30*	0.894	0.025	0.000	0.056	0.155
GP73+AFP-L3	0.925	0.021	0.000	0.884	0.966
GP73+TIP30	0.934	0.019	0.000	0.897	0.972
AFP-L3+TIP30	0.946	0.017	0.000	0.912	0.980
GP73+AFP-L3+TIP30	0.961	0.014	0.000	0.934	0.988

AUROC, area under the receiver operating characteristic curve; Std.e, Standard error; S.A, significance analysis; CI, confidence interval; GP73, Golgi Protein-73; AFP-L3, alpha-fetoprotein-L3' TIP30, Tat-interacting protein-30. a, nonparametric hypothesis; b, null hypothesis; area = 0.5, *TIP30 was negatively correlated with HBV-related cirrhosis, and the AUROC was 0.894.

Table-5: Comparison of the diagnostic value of single and combined detection of serum GP73, AFP-L3 and TIP30 for HBV-related cirrhosis.

Tumor markers	Sensitivity	Specificity	Accuracy
GP73	63.98 (103/161)*#	74.95 (350/467)*#	72.13 (453/628)*#
AFP-L3	60.25 (97/161)*#	78.37 (366/467)*#	72.77 (457/628)*#
TIP30	74.53 (120/161)*	80.73 (377/467)*	79.14 (497/628)*
GP73+AFP-L3	70.19 (113/161)*&	81.15 (379/467)*&	78.34 (492/628)*&
GP73+TIP30	72.05 (116/161)*&	83.30 (389/467)*&	80.41 (505/628)*&
AFP-L3+TIP30	75.78 (122/161)*	84.37 (394/467)*	82.17 (516/628)*
GP73+AFP-L3+TIP30	78.26 (126/161)	86.72 (405/467)	84.55 (531/628)

GP73, Golgi Protein-73; AFP-L3, alpha-fetoprotein-L3' TIP30, Tat-interacting protein-30. Date are expressed as percentage (n/n); *, compared with GP73+AFP-L3+ TIP30, P<0.05; #, compared withTIP30, P<0.05; &, compared with AFP-L3+TIP30, P<0.05.

Table-6: Performance verification of single and combined detection of serum GP73, AFP-L3 and TIP30 for HBV-related cirrhosis.

Tumor markers	Sensitivity		Specificity		Accuracy	
	EG	VG	EG	VG	EG	VG
GP73	64.54±2.30	67.02±3.65*	73.56±2.30	74.35±2.45*	71.98±2.02	72.58±2.65*
AFP-L3	58.46±2.93	60.84±2.05*	78.60±2.98	77.82±2.56*	72.32±2.66	71.80±2.16*
TIP30	73.28±2.66	75.36±3.35*	81.20±2.24	80.86±3.95*	79.40±2.44	80.28±2.69*
GP73+AFP-L3	69.12±2.28	72.54±3.32*	80.46±2.08	82.35±2.84*	77.35±1.98	78.20±2.44*
GP73+TIP30	70.80±2.47	73.35±4.22*	81.90±1.99	83.05±2.50*	80.50±2.40	82.56±2.86*
AFP-L3+TIP30	77.80±2.85	75.64±2.04*	83.40±2.72	84.32±2.38*	83.55±3.03	82.82±3.88*
GP73+AFP-L3+TIP30	77.20±3.65	79.04±4.03*	85.60±3.18	86.05±2.66*	84.50±2.62	83.54±3.35*

GP73, Golgi Protein-73; AFP-L3, alpha-fetoprotein-L3' TIP30, Tat-interacting protein-30. Date are expressed as the mean ± standard deviation; HBV, hepatitis B virus; EG, experimental group; VG, validation group; *, compared with the experimental group, P>0.05.

There were no significant differences in sensitivity, specificity and accuracy between the experimental and the validation groups (Table 6).

The positive detection rate of GP73, AFP-L 3% and TIP30 in HCC patients was significantly higher compared to those with LC, CHB and also compared to the controls

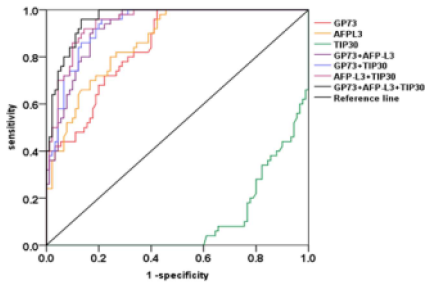

Figure-2: Receiver Opearting Charateristics (ROC) curve analysis of hepatitis B virus (HBV)-related cirrhosis diagnosed by single and combined detection of Golgi Protein-73 (GP73); alpha-fetoprotein-L3 (AFP-L3) and Tat-interacting protein-30 (TIP30). The area under ROC curve of each line is shown in Table 4.

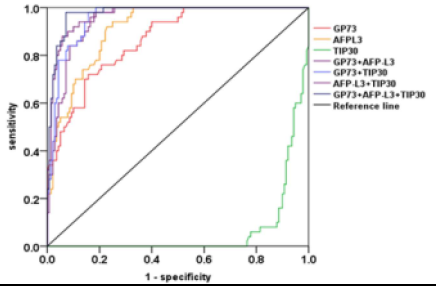

Figure-3: Receiver Opearting Charateristics (ROC) curve analysis of hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC) diagnosed by single and combined detection of Golgi Protein-73 (GP73); alpha-fetoprotein-L3 (AFP-L3) and Tat-interacting protein-30 (TIP30). The area under the ROC curve of each line is listed in Table 8.

Table-7: Comparison among the positive ratios of serum GP73, AFP-L3 and TIP30 in the diagnosis of HBV-related HCC.

Groups	Number (N)	GP73	AFP-L3 (%)	TIP30
HCC	93	76.34 (71/93)*	67.74 (63/93)*	81.72 (76/93)*
LC	161	40.99 (66/161)	39.13 (63/161)	44.10 (71/161)
CHB	271	14.76 (40/271)	12.92 (35/271)	10.70 (29/271)
NC	196	9.69 (19/196)	9.18 (18/196)	3.06 (6/196)
?2		49.307	68.772	89.841
P-value		0.000	0.000	0.000

Data are expressed as positive percentage (n/n). HBV, hepatitis B virus; HCC, hepatocellular carcinoma; LC, liver cirrhosis; CHB, chronic hepatitis B; NC, normal control group; GP73, Golgi Protein-73; AFP-L3, alpha-fetoprotein-L3' TIP30, Tat-interacting protein-30. *, compared with other groups, P<0.01.

Table-8: Comparison of AUROCs of HBV-related cirrhosis diagnosed differently by single and combined detection of GP73, AFP-L3 and TIP30.

Tumor markers	AUROC	Std.ea	S.Ab	95% CI	
				Upper limit	Lower limit
GP73	0.865	0.027	0.000	0.812	0.919
AFP-L3	0.910	0.020	0.000	0.871	0.950
TIP30*	0.064	0.017	0.000	0.032	0.097
GP73+AFP-L3	0.945	0.015	0.000	0.916	0.975
GP73+TIP30	0.957	0.013	0.000	0.931	0.982
AFP-L3+TIP30	0.969	0.011	0.000	0.948	0.990
GP73+AFP-L3+TIP30	0.977	0.009	0.000	0.959	0.995

standard error; S.A, significance analysis; CI, confidence interval; a, non-parametric hypothesis; b, null hypothesis; GP73, Golgi Protein-73; AFP-L3, alpha-fetoprotein-L3' TIP30, Tat-interacting protein-30; area = 0.5, *TIP30 was negatively correlated with HBV-related cirrhosis, and the AUROC was 0.936.

Table-9: Comparison of the diagnostic value of single and combined detection of serum GP73, AFP-L3 and TIP30 for HBV-related HCC.

Tumor markers	Sensitivity	Specificity	Accuracy
GP73	76.34 (71/93)*#	80.09 (503/628)*#	79.61 (574/721)*#
AFP-L3	67.74 (63/93)*#	81.52 (512/628)*#	79.75 (575/721)*#
TIP30	81.72 (76/93)*	83.12 (522/628)*	82.94 (598/721)*
GP73+AFP-L3	78.49 (73/93)*&	85.03 (534/628)*&	84.19 (607/721)*&
GP73+TIP30	79.57 (74/93)*&	86.31 (542/628)*&	85.44 (616/721)*&
AFP-L3+TIP30	83.87 (78/93)*	88.54 (556/628)*	87.93 (634/721)*
GP73+AFP-L3+TIP30	86.02 (80/93)	92.51 (581/628)	91.67 (661/721)

Date are expressed as percentage (n/n); *, compared with GP73+AFP-L3+ TIP30, P<0.05; #, compared withTIP30, P<0.05; &, compared with AFP-L3+TIP30, P<0.05.
HBV, hepatitis B virus; HCC, hepatocellular carcinoma; GP73, Golgi Protein-73; AFP-L3, alpha-fetoprotein-L3' TIP30, Tat-interacting protein-30.

Table-10: Performance verification of single and combined detection of serum GP73, AFP-L3 and TIP30 for HBV-related HCC.

Tumor markers	Sensitivity		Specificity		Accuracy	
	EG	VG	EG	VG	EG	VG
GP73	74.86±2.30	75.02±2.50*	79.26±3.30	80.75±3.45*	79.78±2.82	78.08±3.65*
AFP-L3	66.36±2.58	68.84±2.88*	78.60±2.50	80.24±1.96*	78.05±2.86	77.08±3.56*
TIP30	83.58±2.96	85.36±3.28*	81.55±3.54	82.48±2.65*	82.45±2.04	81.88±2.89*
GP73+AFP-L3	79.52±2.28	80.04±2.32*	84.66±2.88	85.35±3.94*	83.45±2.98	82.18±3.44*
GP73+TIP30	85.80±3.40	83.35±2.82*	85.56±2.99	86.85±2.10*	85.50±2.36	84.86±3.90*
AFP-L3+TIP30	86.40±2.03	85.64±1.88*	87.80±2.80	86.52±3.08*	87.55±3.55	86.50±3.97*
GP73+AFP-L3+TIP30	87.54±3.05	89.04±3.58*	91.60±2.48	90.85±3.46*	91.36±2.77	90.54±3.60*

Date are expressed as the mean ± standard deviation; EG, experimental group; VG, validation group; *, compared with the experimental group, P>0.05. HBV, hepatitis B virus; HCC, hepatocellular carcinoma; GP73, Golgi Protein-73; AFP-L3, alpha-fetoprotein-L3' TIP30, Tat-interacting protein-30.

(Table 7). The diagnostic efficacy of single and combined tests in this regard was also evaluated using ROC curve (Figure 3; Table 8). The single TIP30 test exhibited the best diagnostic efficacy and the diagnostic efficacy of the combined tests was superior to that of any single test (Table 9).

There were no significant differences in sensitivity, specificity and accuracy between the experimental and the validation groups (Table 10).

Discussion

With the high incidence of HBV-related LC and HCC in China, the cost and social burden caused by the diagnosis and treatment of this disease ranks first in the country. Studies have shown that one of the main reasons for the poor prognosis of HCC is the lack of early specific diagnostic indicators and modalities. Imaging examination has certain limitations and cannot be used as a screening method for high-risk population. AFP is the most commonly used tumour marker in HCC, but its sensitivity varies from 33% to 85%, with a mean of 56.3%, which is not optimal for early diagnosis of HCC.[12] During disease progression from HBV infection to CHB, cirrhosis and, finally, liver cancer, there are no simple and high-sensitivity and high-specificity tumour markers which are detectable for the diagnosis, surveillance and evaluation of CHB, LC and HCC. In the current study, based on the analysis of GP73, AFP-L3 and TIP30 serum levels in the CHB, LC and HCC groups, we evaluated the clinical significance of the above-mentioned indicators in the diagnosis of HBV-related LC and HCC.

GP73, AFP-L3 and TIP30 are newly identified tumour markers of HCC. GP73 is a transmembrane protein found in the Golgi body, mainly expressed by bile duct epithelial cells and rarely expressed by hepatocytes. Serum GP73 levels increase due to viral hepatitis or other chronic liver diseases, but are lower compared to those in patients with HCC.[13] According to one study, the sensitivity of GP73 for the diagnosis of liver cancer is 69% and the specificity is 75%, which are superior to those of AFP, and there is a certain correlation with tumor, node, and metastases (TNM) stage of liver cancer.[14] Serum GP73 increased significantly in 57% of liver cancer patients with a low AFP level (<20 μg/l).[15] However, there are also reports that GP73 has no significant correlation with liver cancer.[16] Our study demonstrated that the serum GP73 level and positive detection rate in the LC and HCC groups were significantly higher compared to those in the other groups, and the AUROC curves of GP73 for the diagnosis of HBV-related LC and HCC were 0.846 and 0.865, respectively. The sensitivity and specificity of GP73 single detection for the diagnosis of HBV-related LC and HCC were 63.98% and 74.95%, and 76.34 and 80.09%, respectively. Therefore, the results of the current study support the clinical significance of serum GP73 level for the detection of HBV-related LC and HCC, which may be used to assist early diagnosis.

Due to differences in the glycosylation of AFP in different tissues and organs, it is divided into AFP-L1, AFP-L2 and AFP-L3. AFP-L3 is unique to HCC. Research has revealed that serum AFP-L3 level in HCC patients was significantly higher compared to that in patients with chronic liver disease, and was associated with TNM stage and metastasis.[17,18] However, to the best of our knowledge, there have been no reports on the diagnostic value of AFP-L3 for HBV-related cirrhosis and HBV-related HCC to date. The current study found that serum AFP-L3% and positive detection rate of the LC and HCC groups were significantly higher compared to the other groups, and the sensitivity, specificity and area under curve (AUC) of

AFP-L3% for the diagnosis of HBV-related cirrhosis and HCC were 60.25%, 78.37% and 0.864; and 67.74%, 81.52% and 0.910, respectively. Therefore, the study supports the clinical significance of serum AFP-L3% level for the detection of HBV-related LC and HCC, which was expected to be a better marker for diagnosis, treatment and prognosis compared to AFP.

TIP30 is an expression product of tumour suppressor genes, which was shown to be the same as the expression product of the tumour metastasis suppressor gene CC3 by protein sequence analysis, combining with DNA and ribonucleic acid (RNA) polymerase II to form compounds that regulate cell proliferation, apoptosis and angiogenesis-related gene expression to inhibit tumour occurrence and metastasis.[19] Previous studies reported that TIP30 expression was low in liver cancer, breast cancer, oral haemangioma and small-cell lung cancer, among others. It was further confirmed that the expression of this tumour suppressor gene was often negatively correlated with the occurrence and development of tumours.[20] However, there were also reports that TIP30 is highly expressed in ovarian serous carcinoma, which may be related to the oestrogen receptor.[21] Immunohistochemical (IHC) methods were used to detect the expression of TIP30 in tumour tissues in previous studies;[22,23] by contrast, the ELISA method in the current study was more suitable for the screening of large populations. The present study found that serum TIP30 levels in LC and HCC groups were significantly lower compared to those in the CHB and control groups. During the progression from CHB to LC and HCC, serum TIP30 exhibited a decreasing trend, which was negatively correlated with the occurrence of HCC. The AUROC curves of TIP30 for the diagnosis of HBV-related LC and HCC were 0.894 and 0.936, respectively. The sensitivity, specificity and accuracy of single serum TIP30 detection for the diagnosis of LC and HCC were the best among the three markers tested in the study, with no differences in diagnostic performance between the test and verification groups as determined by random sampling.

Multiple tumour marker detection often has a better diagnostic efficiency. The sensitivity, specificity, accuracy and AUROC curves of combined detection of GP73, AFP-L3% and TIP30 for HBV-related LC were 78.26%, 86.72%, 84.55% and 0.961, respectively, which were better compared to single detection. The sensitivity, specificity, accuracy and AUROCs of combined detection with GP73, AFP-L3% and TIP30 for HBV-related HCC were 86.02%, 92.51%, 91.67% and 0.977 respectively, which were also better compared to single detection. The sensitivity and specificity of AFP-L3% and TIP30 joint detection was the best of all the paired tumour markers in the current study, as determined by cross-validation. Therefore, we recommend AFP-L3% and TIP30 joint detection for early screening in HBV-related LC and HCC patients, which may reduce the cost of testing and also balance the sensitivity and specificity of diagnosis efficiency.

The current study had several limitations, like being a retrospective, single-centre nature and its small sample. Besides, the study included only patients with LC and HCC caused by single HBV infection, and did not include other causes, such as alcohol, other hepatitis virus infections, or with multiple infections of HBV and other types of hepatitis virus. Statistical errors are possible regarding sensitivity, specificity and accuracy because of the small sample size.

Conclusion

In conclusion, we found that higher serum GP73 and AFP-L3%, lower serum TIP30 were in HBV-related cirrhosis and hepatocellular carcinoma patients, suggesting serum GP73 level and AFP-L3% could be increased, serum TIP30 level could be decreased with the progression of HBV-related liver disease. high level of serum GP73 and AFP-L3%, low level of serum TIP30 might be a potential biomarker for screening HCC and LC in HBV infected patients. a combination of three tumour markers including AFP-L3, GP73, and TIP30 showed better accuracy than either marker alone or paired-markers in HBV-related cirrhosis and hepatocellular carcinoma.

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