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Original Research

Serum AFU, GGT and TK1 levels in PHC patients and their correlation with clinicopathology and diagnostic value

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Abstract: To investigate the expression level and clinical significance of fucosidase (AFU), glutamyltranspeptidase (GGT), and thymidine kinase 1 (TK1) in the serum of patients with primary liver cancer (PHC). A total of 135 PHC patients in Baoji Central Hospital from September 2014 to February 2018 were selected as a research group (RG), while 127 healthy subjects were collected as a control group (CG). Enzyme-linked immunosorbent assay (ELISA) was used to detect the AFU, GGT, and TK1 concentrations in serum of the two groups, and the diagnostic value of combined detection of the three for PHC was analyzed. AFU, GGT, and TK1 concentrations in serum of the RG were dramatically higher than those of the CG (P< 0.050). ROC curve analysis showed that the sensitivity of AFU, GGT, and TK1 in the single diagnosis of PHC was 88.00, 94.00, and 66.00% respectively, and the specificity was 68.00, 54.00, and 66.00% respectively. The sensitivity and specificity of the combined diagnosis of PHC were 76.00 and 90.00%, respectively. AFU, GGT, and TK1 concentrations were different in the presence or absence of liver cirrhosis, TNM stage, and tissue type (P< 0.050). AFU, GGT, and TK1 concentrations in PHC patients were dramatically higher than those in healthy people. Combined detection of the three has good diagnostic value for PHC.

Key words: PHC; AFU; GGT; TK1; Diagnostic value; Clinicopathology.

Introduction

Liver cancer is a cancerous growth of hepatocytes, manifested as a mass in the right upper abdomen, with general symptoms such as jaundice and weakness. This cancer is more common in men. Of course, cancer usually metastases to other parts of the body. Primary hepatic carcinoma (PHC) begins with liver cells and is divided into different types according to the type of cancer cells. This type of cancer may be caused by different cells in the liver tissue. The PHC mainly refers to malignant tumors of the liver itself, such as liver cells, cholangiocytes, mesenchymal tissues, etc. According to statistics, liver cancer is the fifth most familiar cancer in the world (1), of which hepatic cellular cancer is one of the most deadly malignant tumors. It is estimated that are 780,000 newly increased PHC cases and 740,000 deaths every year all over the world, and 50% of the total number of cases and deaths is from China (2). The global disease burden caused by cancer has resulted in a large loss of life. Because of its high morbidity, mortality, and aggressiveness, it is still a momentous public health problem in the world.

Due to the high morbidity, mortality, and aggressiveness of PHC, its early diagnosis with serum markers is the focus of research (3-5). Serum markers such as thymidine kinase 1 (TK1), glutamyltranspeptidase (GGT), fucosidase (AFU) have good diagnostic efficacy in various cancers. TK1 (6) phosphorylation is the only way to introduce thymidine into DNA metabolism. Therefore, it is called the pyrimidine salvage pathway enzyme, which is a cell cycle-dependent marker and can be detected in the serum of patients with different types of cancer. GGT (7) mainly comes from the liver and gallbladder system. It is widely distributed in human tissues in the liver and mainly distributed in the liver cytoplasm and epithelium of intrahepatic bile duct. It only increases slightly or moderately in acute hepatitis, chronic active hepatitis and decompensation of liver cirrhosis. Hence, it is widely used as a marker of liver diseases. AFU (8) is a lysosomal acid hydrolytic enzyme, which is mainly involved in the catabolism of various fucosylated glycolipids, glycoproteins, mucopolysaccharides, and other macromolecular substances, and widely exists in lysosomes and body fluids of human tissue cells. It has good sensitivity and a high positive rate in the diagnosis of hepatic cellular cancer and is a useful index for early diagnosis of PHC.

This study aims to analyze the diagnostic value of combined detection of AFU, GGT, and TK1 in PHC patients by detecting their expression levels, and to provide reference and new ideas for clinical diagnosis and treatment of PHC in the future.

Materials and Methods

Data

A total of 135 patients diagnosed with PHC admitted to Baoji Central Hospital from September 2014 to February 2018 were included as research subjects, and prospective analysis was conducted. There were 78 males and 57 females, 35-67 years. During the same period, the blood of 127 healthy subjects was collected as the control group, including 71 males and 56 females, 32-68 years. This experiment has been approved by the Ethics Committee of our Baoji Central Hospital, and all the research patients have signed informed consent forms.

Inclusion and exclusion criteria

Inclusion criteria were as follows: Patients met the diagnostic criteria of PHC (9), diagnosed and treated in Baoji Central Hospital, 30-70 years old, with an educational background of primary school or above, able to cooperate with research. There were no other serious organ diseases affecting the study, and the informed consent forms shall be signed by the patients or their immediate family members. Exclusion criteria were as follows: patients died in the course of treatment, with injury to important organs, complicated with other tumors, other cardiovascular and cerebrovascular diseases, physical disability, prolonged bed rest and unable to take care of themselves, pregnancy, complicated with other autoimmune diseases, other chronic diseases, transferred from one hospital to another, with contraindications to surgery, mental diseases, and language dysfunction, as well as diseases affecting the results of this study.

Methods

A total of 135 PHC patients were regarded as the research group (RG) and 127 healthy subjects as the control group (CG). The RG and the CG were required to draw 5 mL of venous blood respectively on an empty stomach in the morning, and the AFU, GGT, and TK1 levels in each group were detected by enzyme-linked immunosorbent assay (ELISA). AFU test kit was purchased from Shanghai Yiyan Biotechnology Co., Ltd., Article No. (EY-Elisa01). GGT test kit was purchased from Shanghai Yanjin Biotechnology Co., Ltd., Article No. (YOYOBIO-). Test kit TK1 was purchased from Nanjing SenBeiJia Biological Technology Co., Ltd., Article No. (SBJBIO001). The expression levels of AFU, GGT and TK1 serum markers on PHC were detected in strict accordance with the kit instructions.

Blood sample processing

Venous blood was stored at 4℃ for 30 min, serum samples were centrifuged for 10 min (3000 rpm/m), and the supernatant was extracted and stored in a refrigerator at -80℃.

Outcome measures

The expression levels of AFU, GGT, and TK1 in the serum of the RG and the CG were observed and compared, so was the case on the diagnostic value of AFU, GGT, and TK1 single index detection and their combined detection for PHC and differential expression levels in different pathological characteristics of PHC.

Statistical methods

All data were processed and analyzed by SPSS 24.0 software system (Beijing Strong-vinda Information Technology Co., Ltd.). All graphics were drawn by Graphpad8 (Shenzhen Qiruitian Software Technology Co., Ltd.) software and the results were checked twice. The counting data were tested by x^2 test, and the measurement data were tested by t, expressed by mean±standard deviation (x±s). ROC curve was used to evaluate diagnostic efficiency, calculate sensitivity, specificity, etc. $P< 0.050$ has statistical significance.

Results

Comparison of clinical data

Comparing the clinical data of the RG and the CG in terms of age, gender, height, weight, marital status, nationality, place of residence, smoking and exercise, it was found that there was no significant difference between the two groups ($P> 0.050$), and there were statistical differences in drinking history, APF, CEA, CA199 and CA125 classification between the two groups ($P< 0.050$), as shown in Table 1.

Expression levels of Afu, Ggt and Tk1 in serum of patients in the Rg and healthy subjects in the Cg

AFU, GGT, and TK1 were (15.23±8.72) U/L, (25.08±10.15) U/L and (0.76±1.05) pmol/L respectively in the serum of healthy subjects in the CG. AFU, GGT, and TK1 in the serum of patients in the RG were (31.35±17.26) U/L, (62.72±25.51) U/L, (3.78±3.45) pmol/L respectively. The three serum indexes in the CG were lower than those in the RG ($P< 0.050$), as shown in Table 2.

Diagnostic efficacy of AFU, GGT and TK1 for PHC

ROC curve analysis revealed that when the cut-off value was 27.39 U/L, the sensitivity, specificity, and AUC of AFU for a single diagnosis of PHC were 88.00, 68.00, and 0.825 respectively. When the cut-off value was 40.96 U/L, the sensitivity, specificity and AUC of GGT in a single diagnosis of PHC were 94.00, 54.00, and 0.713% respectively. When the cut-off value was 1.19pmol/L, the sensitivity, specificity, and AUC of TK1 single diagnosis PHC were 66.00, 84.00, and 0.768% respectively. However, taking AFU, GGT, and TK1 as independent variables, Logistic regression analysis was carried out, and the combined formula LOG (P)= 1.621+0.522× AFC+0.812× GGT+0.214× TK1

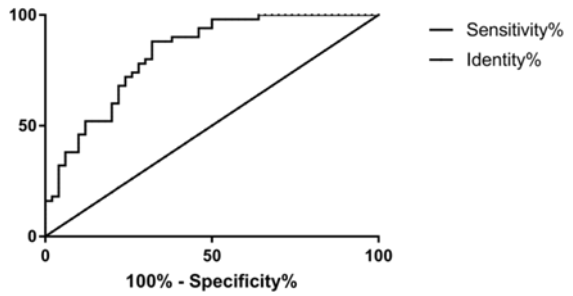

Figure 1. ROC curve analysis of AFU. The cut-off value, AUC, Std. Error, 95%5CI, sensitivity and specificity of AFU in diagnosing PHC were 27.39 U/L, 0.825, 0.041, 0.744-0.905, 88.00, and 68.00% ($P< 0.001$).

Table 1. Comparison of clinical data between the two groups [n(%)].

	Research Group (RG) (135)	Control group (CG) (127)	x^2 or t	*P*
Age (years)	51.36±9.68	52.29±10.24	0.756	0.450
Gender			0.094	0.760
Male	78 (57.78)	71 (55.91)		
Female	57 (42.22)	56 (44.09)		
Weight (KG)	62.67±8.64	63.31±9.15	0.582	0.561
Height (cm)	171.26±3.28	170.82±4.15	0.955	0.341
Marital status			1.236	0.266
Married	128 (94.81)	116 (91.34)		
Unmarried	7 (5.19)	11 (8.66)		
Nationality			0.088	0.766
Han	113 (83.70)	108 (85.04)		
Ethnic minorities	22 (16.30)	19 (14.96)		
Place of residence			0.044	0.833
Cities and towns	92 (68.15)	85 (66.93)		
Countryside	43 (31.85)	42 (33.07)		
Smoking history			0.026	0.871
Yes	72 (53.33)	69 (54.33)		
No	63 (46.67)	58 (45.67)		
Drinking history			47.71	0.001
Yes	112 (82.96)	53 (41.73)		
No	23 (17.04)	74 (58.27)		
Exercise habits			1.915	0.166
Yes	65 (48.15)	72 (56.69)		
No	70 (51.85)	55 (43.31)		
APF (IU/mL)	78.35±9.47	1.09±1.03	91.42	0.001
CEA (ng/mL)	23.52±7.19	2.26±1.55	32.61	0.001
CA199 (U/mL)	31.32±8.25	3.42±1.67	37.39	0.001
CA125 (U/mL)	35.61±7.93	2.47±1.13	46.65	0.001

Table 3. Diagnostic efficiency of AFU, GGT and TK1 on PHC.

Group	Number of cases	AFU (U/L)	GGT (U/L)	TK1 (pmol/L)
Research group (RG)	135	31.35±17.26	62.72±25.51	3.78±3.45
Control group (CG)	127	15.23±8.72	25.08±10.15	0.76±1.05
t value		9.451	15.51	9.460
p-value		0.001	0.001	0.001

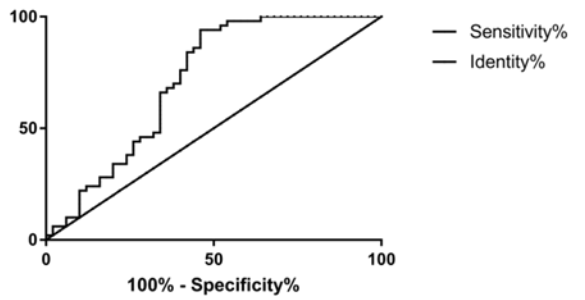

Figure 2. ROC curve analysis of GGT. The cut-off value, AUC, Std. Error, 95%5CI, sensitivity and specificity of GGT in diagnosing PHC were 40.96 U/L, 0.713, 0.053, 0.609-0.818, 94.00, and 54.00%, respectively (P< 0.001).

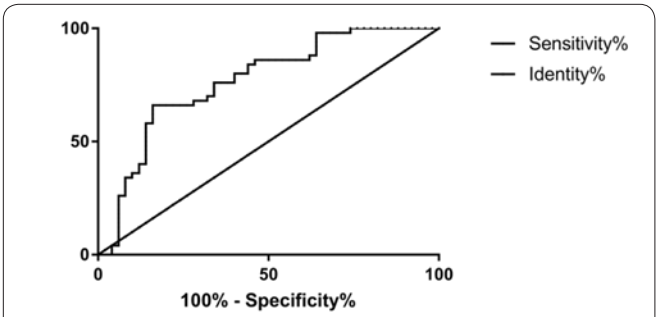

Figure 3. ROC curve analysis of TK1. The cut-off value, AUC, Std. Error, 95%5CI, sensitivity and specificity of TK1 in diagnosing PHC were 1.19 pmol/L, 0.768, 0.048, 0.673-0.861, 66.00, and 84.00%, respectively (P< 0.001).

was obtained. When the cut-off value was 0.36, the sensitivity, specificity and AUC of the combined formula for diagnosing PHC were 76.00, 90.00 and 0.962% respectively. More details were shown in Table 3 and Figures 1-4.

Correlation between Afu, Ggt, Tk1 and different Clinical pathological features of Phc

The AFU, GGT and TK1 levels in the RG had no significant difference in a different age, gender, course of the disease, number of tumors and classification of liver cirrhosis (P> 0.050). AFU, GGT, and TK1 concen-

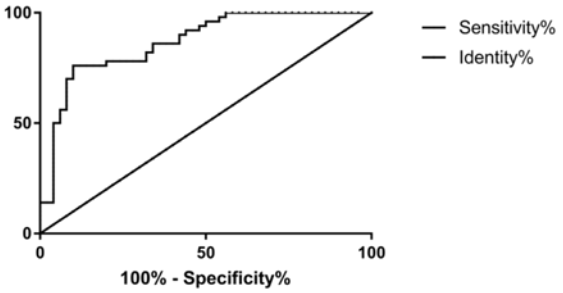

Figure 4. ROC curve analysis chart of a three combined diagnosis. The cut-off value, AUC, Std. Error, 95%5CI, sensitivity and specificity of TK1, GGT, and AFU combined diagnosis in PHC were 0.36, 0.867, 0.360, 0.797-0.938, 76.00, and 90.00%, respectively (P< 0.001).

Table 4. Correlation between AFU and PHC different pathological features clinically (U/L).

	n (135)	AFU	t	P
Age (years)			0.780	0.437
>50	70	30.17±15.32		
≤50	65	32.28±16.13		
Gender			0.196	0.845
Male	78	30.68±16.27		
Female	57	31.25±17.22		
Course of disease			0.389	0.698
>5	82	30.42±16.18		
≤5	53	31.56±17.33		
Number of tumors			0.283	0.777
Single	37	30.69±16.92		
Multiple	98	31.63±17.29		
Cirrhosis			2.074	0.040
Yes	42	36.89±18.37		
No	93	30.29±16.53		
TNM stage			2.379	0.019
Stages I-II	56	31.13±17.01		
Stages III-IV	79	38.35±17.62		
Tissue type			2.094	0.038
Poorly differentiated	81	37.26±17.47		
Moderately and highly differentiated	54	30.92±16.88		

trations were various from those of liver cirrhosis, TNM stage and tissue type (P< 0.050), as shown in Table 4-6.

Discussion

PHC is one of the common malignant tumors in the world, and its death toll accounts for 9.1% of all cancer deaths (10). According to Petrick J L et al. (11), it is the fifth most common cancer among men and the second most common cause of cancer death in the world. The ratio of mortality to the morbidity of liver cancer is 0.95, indicating poor prognosis. As the etiology and pathogenesis of PHC have not yet been determined, some studies have shown that (12-14) the diagnosis of PHC by serum factors is more reliable and the detection method

Table 5. Correlation between GGT and PHC different pathological features clinically (U/L).

	n (135)	GGT	t	P
Age (years)			0.079	0.937
>50	70	62.23±25.18		
≤50	65	61.89±24.92		
Gender			0.266	0.790
Male	78	61.35±24.89		
Female	57	62.51±25.15		
Course of disease			0.299	0.766
>5	82	60.89±26.18		
≤ 5	53	62.25±25.31		
Number of tumors			0.196	0.845
Single	37	61.75±25.19		
Multiple	98	62.71±25.49		
Cirrhosis			2.153	0.033
Yes	42	70.21±28.08		
No	93	60.35±22.93		
TNM stage			2.531	0.020
Stages I-II	56	60.56±24.39		
Stages III-IV	79	71.29±27.31		
Tissue type			2.158	0.037
Poorly differentiated	81	69.98±26.75		
Moderately and highly differentiated	54	60.08±25.13		

Table 6. Correlation between TK1 and PHC different pathological characteristics clinically (pmol/L).

	n (135)	TK1	t	P
Age (years)			0.837	0.404
>50	70	3.62±3.38		
≤50	65	3.15±3.13		
Gender			0.717	0.474
Male	78	3.43±3.41		
Female	57	2.98±3.85		
Course of disease			0.324	0.746
>5	82	3.54±3.26		
≤ 5	53	3.35±3.42		
Number of tumors			0.259	0.796
Single	37	3.52±3.08		
Multiple	98	3.69±3.51		
Cirrhosis			3.347	0.001
Yes	42	4.92±3.49		
No	93	2.95±3.01		
TNM stage			3.292	0.001
Stages I-II	56	3.01±3.13		
Stages III-IV	79	4.98±3.62		
Tissue type			2.648	0.008
Poorly differentiated	81	4.81±3.42		
Moderately and highly differentiated	54	3.19±3.46		

is convenient. Hence, it is extremely important to find an index that can accurately reflect the occurrence, progression, and change of PHC and has convenient detection means. In this study, the expression levels of AFU, GGT, and TK1 in PHC patients were detected to contribute to the early screening of PHC.

The results of this experiment showed that the concentrations of AFU, GGT, and TK1 in the serum of PHC patients were higher than those of healthy subjects. This was approximately the same as the research results of MAO et al. (15), Yang et al. (16), and Xie et al. (17), which could prove the experimental results, and this study further studied the effectiveness of the combined diagnosis of PHC by the three. TK1 was first used to diagnose breast cancer system tumors, but with the deepening of research, it was found to be not only relevant to breast cancer, but also had abnormal expression in gastric cancer, colorectal cancer, ovarian cancer and other malignant tumors (18-20). It is a widely proven serum biomarker and is also a universal marker for diagnosing cancer. As a fully characterized serum biomarker, it has been established as a tumor biomarker. It increases in a phased manner and increases with disease progression (21).

In this study, serum TK1 of PHC patients is higher than that of healthy people and increases gradually with the progression of tumors in PHC. Wang et al. (22) explored that serum TK1 was more reliable than CEA and AFP in diagnosing cancer, which also indicated the value of TK1 in diagnosing PHC in this study. GGT is a biomarker of liver cancer, but Geng et al. (23) confirmed that it was considered to be the best liver cancer marker except for AFP. In this research, the GGT concentration in PHC patients was detected, and it was found that the concentration of GGT in PHC patients was higher than that in healthy people. It can also be confirmed that GGT has important significance in PHC. Zhu et al. (24) discovered that AFU is widely present in human cells, and the serum AFU level of healthy people is relatively low. As AFU inhibitors produced by hepatic cellular cancer reduce the hydrolysis ability of substrates, bringing about substrate accumulation and an increase of AFU level, the serum AFU level of liver cancer patients increases. According to ROC curve analysis, AFU's diagnostic efficiency of PHC was remarkably higher than that of healthy people and AUC was higher than 0.7, which showed AFU's diagnostic efficiency of PHC was better. Zhu et al. (24) pointed out that AFU's AUC for PHC diagnosis was 0.78, which was also consistent with our research results. AFU, GGT, and TK1 combined detection have been found to have better diagnostic efficiency for PHC diagnosis, indicating that it can be used as a screening index for PHC in the future, which is line with the conclusion that AFU combined with other cancer markers for detection has higher diagnostic value for tumors (25-46).

This experiment aims to explore the expression levels of AFU, GGT, TK1, and other serum markers in patients with PHC and the efficacy of their combined diagnosis of PHC. However, due to limited conditions, there are still limitations. For example, the test cycle is short, and targeted drugs and cell-based experiments cannot be conducted. Currently, there are few studies on the combined diagnosis of PHC by AFU, GGT and TK1, therefore the comparison with more experimental results could not be made. A more in-depth experimental analysis will be conducted in the future to provide a reference for clinical practice. To summarize, the concentrations of AFU, GGT, and TK1 in PHC patients are obviously higher than those in healthy people. AUC of the three serum markers is higher than 0.7 through ROC curve analysis, which indicates that the three serum markers in this study have good diagnostic efficacy. AUC of the three combined diagnoses is as high as 0.867, which means that the combined detection of AFU, GGT, and TK1 has better diagnostic efficiency for PHC and is expected to become an excellent indicator for its clinical diagnosis.

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