INDIVIDUAL AND COMBINED DIAGNOSTIC VALUES OF THE SERUM TUMOR MARKERS α-FETOPROTEIN, THYMIDINE KINASE 1, AND MIR-202 FOR PRIMARY HEPATIC CARCINOMA

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ABSTRACT

Objectives*: To investigate the individual and combined diagnostic values of the serum tumor markers α-fetoprotein (AFP), thymidine kinase 1 (TK1), and micro RNA (miR)-202 for primary hepatic carcinoma.*

Methods: *A total of 48 patients with primary liver cancer (PCL), who were treated in our hospital from July 2018 to July 2019, were recruited for the PLC group, and 52 patients with hepatitis were recruited for the hepatitis group. Thirty healthy individuals, who were examined at the examination center of our hospital, were selected as the control group. The levels of AFP, TK1, and miR-202 were detected and compared among groups. The diagnostic values for AFP, TK1, and miR-202 for PLC were analyzed by performing receiver operating characteristic (ROC) curve analysis.*

Results: *Serum AFP and TK1 levels in patients with PLC were significantly higher than those in the hepatitis and the control groups (P<0.01). Serum AFP and TK1 levels for the hepatitis group were significantly higher than those for the control group (P<0.01). Serum miR-202 levels in the PLC group were significantly lower than those in the hepatitis and control groups (P<0.01), and serum miR-202 levels in the hepatitis group were significantly lower than those in the control group (P<0.01). AFP levels were positively correlated with TK1 levels (r = 0.243, P = 0.024) and negatively correlated with miR-202 levels (r = –0.126, P = 0.016). TK1 levels were negatively correlated with miR-202 levels (r = –0.247, P = 0.018). The ROC curve analysis revealed that the area under the ROC curve (AUC) for the ability of AFP to diagnose PLC was 0.906, with the best predictive value determined to be 264.81 ng/mL, which had a sensitivity of 89.16% and a specificity of 81.21%. The AUC for the diagnostic capability of TK1 for PLC was 0.899, with the best predictive value determined to be 4.58 pmol/L, which had a sensitivity of 81.40% and a specificity of 82.84%. The AUC for miR-202 to diagnose PLC was 0.838, with the best predictive value determined to be 0.41, which had a sensitivity of 85.18% and a specificity of 78.43%. The combination of AFP, TK1, and miR-202 diagnosed PLC with an AUC of 0.923, a sensitivity of 90.12%, and a specificity of 91.46%. The combination of AFP, TK1, and miR-202 had the highest diagnostic value for PLC.*

Conclusions: *AFP, TK1, and miR-202 are expressed at abnormal levels in the serum of patients with PLC, and the individual or combined detection of these molecules can be valuable for the diagnosis of PLC, with the combination of AFP, TK1, and miR-202 presenting the highest diagnostic value for PLC.*

Keywords: *α-fetoprotein, thymidine kinase 1, miR-202, diagnosis, primary hepatic carcinoma.*

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Introduction

Primary hepatic carcinoma (PLC) is one of the most common malignant tumors. Cirrhosis, genetics, environmental factors, alcoholism, chemical carcinogens, and viral hepatitis can cause PLC, and clinical symptoms, such as fatigue, liver pain, loss of appetite, weight loss, and enlarged liver area have been associated with PLC[(1)]. The incidence of PLC can be difficult to detect, which allows the disease to progress rapidly, resulting in a high degree of deterioration and a high mortality rate. A survey found that PLC is the fifth most common malignant tumor, worldwide, with the second-highest mortality rate[(2)]. In China, PLC has a high incidence, especially in the southeast coastal areas, which seriously harms people's health[(3)]. Because the clinical symptoms of PLC are not obvious in the early stage, most patients

have progressed to middle and advanced stages by the time PLC is diagnosed, and the prognosis is poor. Patients who receive targeted treatment have a higher probability of relapse within 5 years[4]. Therefore, early detection and early treatment are important for PLC prognosis. Alpha-fetoprotein (AFP) and thymidine kinase 1 (TK1) can affect the occurrence and development of PLC. Clinically, these proteins are utilized as serum markers of PLC, to diagnose and monitor PLC; however, they are less sensitive to early and micro PLCs[5, 6].

Micro RNAs (miRNAs) are a class of endogenous, non-coding, small RNAs. Studies have found that the abnormal expression of miRNAs is closely associated with tumor occurrence, indicating that miRNAs may assist with the diagnosis of malignant tumors[7]. The serum level of miR-202 in patients with PLC is lower than that in the normal population, which may be related to PLC[8]. We aimed to study the value of the serum tumor markers AFP, TK1, and miR-202 for the diagnosis of PLC, either alone or in combination.

Materials and methods

Materials

A total of 48 PLC patients, who were treated in our hospital from July 2018 to July 2019, were recruited for the PLC group.

The PLC group inclusion criteria were as follows:

• Patients met the PLC diagnostic criteria defined in the 2015 edition of the Guidelines for the Diagnosis and Treatment of Primary Liver Cancer Standard Pathological Diagnosis Guidelines;

• Patients were treated for the first time and did not receive any tumor treatments before being admitted to our hospital;

• And surgical indications were identified.

The exclusion criteria were as follows:

• Individuals with mental illness or language communication impairments;

• PLC combined with other malignant tumors;

• The appearance of metastatic liver cancer;

• Diagnosis with an autoimmune disease;

• Pregnant women;

• And patients with drug-induced liver disease or viral liver disease.

The PLC group contained 48 patients, including 26 men and 22 women. The mean age was 51.42±9.45 years, and the mean body mass index (BMI) value was 20.23±1.18 kg/m^2. Patients in the hepatitis group were diagnosed with hepatitis according to clinical manifestations, laboratory tests, and imaging examinations, without liver mass lesions, liver malignant tumors, and other serious organ diseases. The hepatitis group contained 52 patients, including 27 men and 25 women. The mean age was 51.33±10.35 years, and the mean BMI value was 20.16±1.12 kg/m^2. Healthy individuals, who were examined at the physical examination center of our hospital, were selected for the control group.

The inclusion criteria for the control group were as follows:

• No hepatitis virus carriers;

• Blood tests, liver function, and kidney function were normal;

• Chest computed tomography (CT) and liver, gallbladder, and pancreas ultrasound examinations were normal;

• And no other serious organ diseases were identified.

The control group contained 30 individuals, including 16 men and 14 women, aged 50.45±0.24 years, with a mean BMI value of 20.20±0.98 kg/m^2. No significant differences in age, sex, and BMI were observed among the subjects in each group (P>0.05). The results are shown in Table 1.

Group	Age (years)	Gender (n)		BMI value (kg/m^2)
		Men	Women	
Control group (n = 30)	50.45±10.24	16	14	20.20±0.98
Hepatitis group (n = 52)	51.33±10.35	27	25	20.16±1.12
PLC group (n = 48)	51.42±9.45	26	22	20.23±1.18
F/χ^2	0.100	0.052		0.050
P	0.906	0.975		0.952

Table 1: Comparison of general information for the subjects in each group ($\bar{x}\pm s$).
BMI, body mass index; PLC, primary liver cancer.

Observation indicators

A 10-ml sample of fasting venous blood was collected from each subject in each group and centrifuged, at 3,500 r/min for 10 minutes.

The upper serum was carefully separated and stored at –80°C, to avoid repeated freeze-thaw cycles. The chemiluminescence method was used to detect the serum AFP level in each sample, and the enzyme immunoblotting chemiluminescence method was used to detect the serum TK1 level in each sample. The serum miR-202 levels in each sample were detected by fluorescent quantitative polymerase chain reaction (PCR). The levels of AFP, TK1,

and miR-202 were compared between groups, and correlations between AFP, TK1, and miR-202 were studied. The diagnostic values of AFP, TK1, and miR-202 for PLC, either alone or combined, were analyzed.

Statistical methods

The data collected during this study were analyzed using the software package SPSS, v 20.0. All measurements were expressed as ($\bar{x}\pm s$), and comparisons between groups were tested by the F test. Count data were expressed as percentages, and comparisons between groups were tested using the χ^2 test. Grade data comparisons were performed using the Ridit test. The ROC curve was used to analyze the diagnostic values of AFP, TK1, and miR-202, either alone or in combination, for PLC. Results were considered significant at P<0.05.

Results

Comparisons of serum AFP, TK1, and miR-202 levels

The serum AFP and TK1 levels in patients in the PLC group were significantly higher than those in the hepatitis and control groups (P<0.01). The serum AFP and TK1 levels of patients in the hepatitis group were significantly higher than those in the control group (P<0.01).

The serum level of miR-202 in the PLC group was significantly lower than those in the hepatitis and control groups (P<0.01). The serum level of miR-202 was significantly lower in the hepatitis group than in the control group (P<0.01). The results are shown in Table 2.

Group	AFP (ng/mL)	TK1 (pmol/L)	miR-202
Control group (n = 30)	11.46±5.48	0.82±0.19	1.42±0.43
Hepatitis group (n = 52)	54.16±11.19[a]	1.46±0.64[a]	0.56±0.18[a]
PLC group (n = 48)	314.41±56.40[ab]	5.43±1.84[ab]	0.32±0.19[ab]
F	947.12	223.8	170.46
P	<0.001	<0.001	<0.001

Table 2: Comparisons of serum AFP, TK1, and miR-202 levels ($\bar{x}\pm s$).
Note: [a]P<0.05 compared with the control group. [b]P<0.05 compared with the hepatitis group. AFP, alpha-fetoprotein; TK1, thymidine kinase 1; miR, micro RNA; PLC, primary liver cancer.

Correlation analyses among AFP, TK1, and miR-202 levels

AFP levels were positively correlated with TK1 levels (r = 0.243, P = 0.024) and negatively correlated with miR-202 levels (r = –0.126, P = 0.016). TK1 levels were negatively correlated with miR-202 levels (r = –0.247, P = 0.018). The results are shown in Table 3.

index	AFP		TK1		miR-202	
	r	*p*	*r*	*p*	*R*	*p*
AFP	-		0.243	0.024	–0.126	0.016
TK1	0.243	0.024	-		–0.247	0.018
miR-202	–0.126	0.016	–0.247	0.018	-	

Table 3: Correlation analyses among AFP, TK1, and miR-202 levels.
AFP, alpha-fetoprotein; TK1, thymidine kinase 1; miR, micro RNA.

Individual or combined diagnostic value of AFP, TK1, and miR-202 for primary hepatic carcinoma

The ROC curve analysis showed that the AUC for the AFP diagnosis of PLC was 0.906, and the best predictive value was 264.81 ng/mL, which had a sensitivity was 89.16% and a specificity of 81.21%.

The AUC of TK1 for the diagnosis of PLC was 0.899, and the best predictive value was 4.58 pmol/L, which had a sensitivity of 81.40% and a specificity of 82.84%. The AUC for the miR-202 diagnosis of PLC was 0.838, and the best predictive value was 0.41, which had a sensitivity of 85.18% and a specificity of 78.43%.

The combination of AFP, TK1, and miR-202 for the diagnosis of PLC had an AUC of 0.923, a sensitivity of 90.12%, and a specificity of 91.46%. The combination of AFP, TK1, and miR-202 had the highest diagnostic value for PLC. The results are shown in Table 4 and Figure 1.

Index	AUC	95% CI	Best predictive value	Sensitivity	Specificity
AFP	0.906	0.846-0.956	264.81	89.16%	81.21%
TK1	0.899	0.861-0.943	4.58	81.40%	82.84%
miR-202	0.838	0.813-0.945	0.41	85.18%	78.43%
AFP+FK1 +miR-202	0.923	0.892-0.978	-	90.12%	91.46%

Table 4: Individual and combined diagnosis value for AFP, TK1, and miR-202 for primary hepatic carcinoma.
AUC, area under the receiver operating characteristic curve; 95% CI, 95% confidence interval; AFP, alpha-fetoprotein; TK1, thymidine kinase 1; miR, micro RNA.

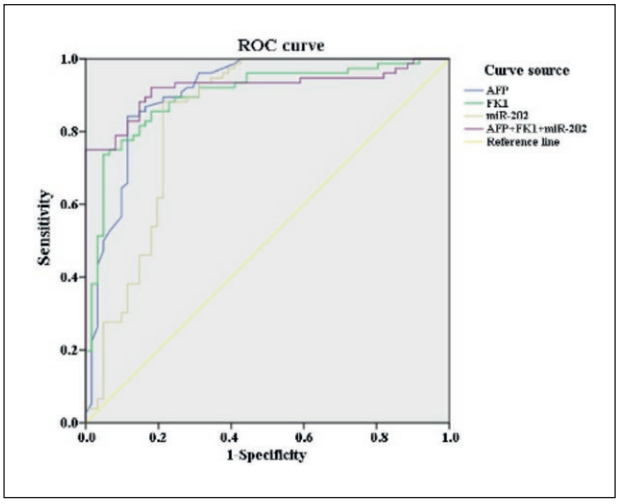

Figure 1: Individual and combined diagnostic values for AFP, TK1, and miR-202 for primary hepatic carcinoma.

Discussion

The occurrence and development of PLC have been associated with a variety of factors. Because the early onset of the disease can be difficult to detect, only a few patients are diagnosed and treated during the early stages, when the prognosis for PLC is often extremely optimistic[8]. Clinically, PLC is diagnosed primarily through imaging and liver biopsy; however, this procedure can be very traumatic and can only be performed on patients in healthy conditions[9]. B-ultrasound and CT can result in large errors, and with a high probability of missed diagnosis[10]. Therefore, identifying novel methods for screening PLCs is very important. AFP is a glycoprotein that is synthesized by fetal liver cells and yolk sacs and is known to regulate growth and development, induce T-lymphocyte apoptosis, and play an important role in transportation and immunity. Serum AFP levels are generally low in healthy adults and increase in patients with hepatitis and cirrhosis, with very high levels of expression in patients with PLC. AFP has been used as a positive detection indicator for various tumors in the clinic[11]. In this study, the serum AFP level of patients in the PLC group was significantly higher than those in the hepatitis and control groups, and the serum AFP level of patients in the hepatitis group was significantly higher than that in the control group (P<0.01). These results suggest that the changes in AFP levels are closely related to the incidence of PLC.

TK1 is a key enzyme involved in the synthesis of DNA and can regulate cell proliferation[12]. TK1 shows low or no expression in the serum of healthy people, whereas strong expression levels can be detected in the serum of patients with tumor proliferation. Therefore, TK1 can be used as a serological test index to screen malignant tumor proliferative lesions and to evaluate the prognosis after treatment[13].

In this study, the serum TK1 level of patients in the PLC group was significantly higher than those of the hepatitis and control groups, and the serum TK1 level of patients in the hepatitis group was significantly higher than that in the control group (P<0.01). These results suggested that the tumors of PLC patients proliferate in a large area, and TK1 may be useful for screening tumor incidence.

Numerous studies have shown that a variety of miRNAs are abnormally expressed in the serum of patients with malignant tumors. miRNAs are stable and not easily degraded and have great significance during tumor screening[14]. miR-202 is a type of miRNA that has been reported to be abnormally expressed in tumor patients, including those with brain tumors, uterine tumors, gastric cancers, and a few reports in PLC patients[15]. In this study, the serum miR-202 levels identified in the PLC group were significantly higher than those in the hepatitis and control groups, and the serum miR-202 level was significantly higher in the hepatitis group than that in the control group (P <0.01). These results suggested that miR-202 could play a role in the suppression of oncogenes and may inhibit the development of PLC.

The ROC curve analysis found that the AUC of AFP for the diagnosis of PLC was 0.906, and the best predictive value was 264.81 ng/mL, which had a sensitivity of 89.16% and a specificity of 81.21%. The AUC of TK1 for the diagnosis of PLC was 0.899, and the best predictive value was 4.58 pmol/L, which had a sensitivity of 81.40% and a specificity of 82.84%. The AUC of miR-202 for the diagnosis of PLC was 0.838, and the best predictive value was 0.41, which had a sensitivity of 85.18% and a specificity of 78.43%.

The combination of AFP, TK1, and miR-202 for the diagnosis of PLC had an AUC of 0.923, a sensitivity of 90.12%, and a specificity of 91.46%. These results demonstrated that the combination of AFP, TK1, and miR-202 showed the highest diagnostic value for PLC and may play a greater role in the evaluation of PLC during clinical practice. In addition, AFP levels were positively correlated with TK1 levels and negatively correlated with miR-202 levels, whereas TK1 levels were negatively correlated with miR-202 levels, indicating the existence of a relationship among the serum levels of AFP, TK1, and miR-202.

In summary, the serum AFP and TK1 levels of PLC patients were significantly higher than those of healthy people, whereas the levels of miR-202 were lower in PLC patients than those in healthy people. All three serum markers have diagnostic value for PLC, with the combination of all three markers displaying the highest diagnostic value for PLC, which could improve the accuracy PLC diagnosis. However, this study utilized a small sample size and a short period of time, and the serum levels of these markers in PLC patients with different prognoses have not been evaluated. The sample size and time course should be expanded in future studies to explore the levels of these markers in patients with different prognoses.

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