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Evaluation of the diagnostic value of alpha-L-fucosidase, alpha-fetoprotein and thymidine kinase 1 with ROC and logistic regression for hepatocellular carcinoma

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ABSTRACT

The purpose of this study was to evaluate the diagnostic efficiency for hepatocellular carcinoma (HCC) with the combined analysis of alpha-L-fucosidase (AFU), alpha-fetoprotein (AFP) and thymidine kinase 1 (TK1). Serum levels of AFU, AFP and TK1 were measured in: 116 patients with HCC, 109 patients with benign hepatic diseases, and 104 normal subjects. The diagnostic value was analyzed using the logistic regression equation and receiver operating characteristic curves (ROC). Statistical distribution of the three tested tumor markers in every group was non-normally distributed (Kolmogorov–Sminov test, $Z = 0.156–0.517$, $P < 0.001$). The serum levels of AFP and TK1 in patients with HCC were significantly higher than those in patients with benign hepatic diseases (Mann–Whitney U test, $Z = -8.570$ to -5.943, all $P < 0.001$). However, there was no statistically significant difference of AFU between these two groups (Mann–Whitney U test, $Z = -1.820$, $P = 0.069$). The levels of AFU were significantly higher in patients with benign hepatic diseases than in normal subjects (Mann–Whitney U test, $Z = -7.984$, $P < 0.001$). Receiver operating characteristic curves (ROC) in patients with HCC versus those without HCC indicated the optimal cut-off value was 40.80 U/L for AFU, 10.86 μg/L for AFP and 1.92 pmol/L for TK1, respectively. The area under ROC curve (AUC) was 0.718 for AFU, 0.832 for AFP, 0.773 for TK1 and 0.900 for the combination of the three tumor markers. The combination resulted in a higher Youden index and a sensitivity of 85.3%. The combined detection of serum AFU, AFP and TK1 could play a complementary role in the diagnosis of HCC, and could significantly improve the sensitivity for the diagnosis of HCC.

1. Introduction

Despite progress made being during the past few decades, HCC is still one of the most frequent and deadly cancers worldwide in both men and women. In the United States, HCC ranks as having the fifth highest mortality in males, with an estimated 14,890 death cases and ranked ninth in females, with an estimated 6780 death cases, according to the 2013 report by the United States cancer society [1]. Globally, there are approximately 750,000 new cases of liver cancer reported each year, 70–85% of which are HCC [2,3]. Due to the asymptomatic nature of an early case of HCC and lack of effective diagnostic and screening strategies, most patients (>80%) are presented with the apparent advanced stage of HCC [4]. It is well known that the prognosis of HCC is poor. Therefore, the prevention of HCC is a significant public health issue. Early detection for HCC is of the utmost importance [5]. Detection of a tumor biomarker is effective and a common approach to screen HCC because it is convenient, non-invasive and inexpensive with a high degree of accuracy.

Alpha-L-fucosidase (AFU) is a liposomal enzyme widely present in all mammalian cells, blood and body fluid. It can be found in the serum of healthy adults. The activity of this liposomal enzyme is detectable and elevated activities are observed in the sera of HCC patients compared with chronic liver disease and healthy individuals [6,7].

Abbreviations: AFP, alpha-fetoprotein; AFU, alpha-L-fucosidase; AUC, area under receiver operating characteristic curve; CI, confidence interval; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HDV, hepatitis D virus; HEV, hepatitis E virus; HIV, human immunodeficiency virus; ROC, receiver operating characteristic curve; RPM, rotation per minute; SE, standard error; TK1, thymidine kinase 1

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Alpha-fetoprotein (AFP) is a glycoprotein secreted by the fetal liver and yolk sac during prenatal development. The fetal liver becomes the main site of AFP synthesis as the yolk sac degenerates after 12 weeks of gestation [8]. AFP levels are usually high at birth and decrease to adult levels within the first year of life. In healthy pregnant women AFP may reach concentrations of 250 mg/L. After birth, these concentrations fall rapidly [9]. AFP is widely used as a tumor biomarker in the early diagnosis of HCC.

TK1 is a diagnostic biomarker for a variety of tumor types, which is involved in DNA repair and is primarily elevated during S phase. TK1 is associated with proliferating cells [10]. A clinical investigation [11] performed on 11,880 people within China during 2005 to 2007 showed that serum level of TK1 with values >2.0 pmol/L may indicate an early risk for the development of malignancies later in life.

Today, lots of tumor biomarkers have been conducted as a complement or substitute for AFP in order to improve sensitivity and specificity in diagnosing HCC. The aim of the present study is to assess the diagnostic value of joint detection of AFU, AFP and TK1 in the diagnosis for HCC.

2. Material and methods

We retrospectively analyzed the clinic pathologic data of patients with HCC and benign liver disorders at the Department of Oncology in Affiliated Fuding Hospital, Fujian University of Traditional Chinese Medicine in China between January 2012 and November 2014. They included a group of 116 patients with HCC and a group of 109 patients with benign liver diseases. Additionally, a group of 104 healthy blood specimens from routine check-ups within the same hospital were used as controls at the same time. There were 75 males and 41 females with a mean age of 57.8 ± 11.3 years (range, 27–79 years) in HCC group, 68 males and 41 females with a mean age of 54.1 ± 11.9 years (range, 32–84 years) in benign liver disease group and 70 males and 34 females with a mean age of 56.9 ± 16.0 years (range, 22–85 years) in normal control group, respectively.

Diagnosis for HCC was based on clinical investigations, which included laboratory tests, along with radiological imaging in selected cases. After hepatic resection, two pathologists confirmed the diagnosis of HCC conducted histopathological examination of the tissue specimens. Diagnosis of benign liver disease was based on liver histology as well as laboratory and imaging evidence of hepatic decomposition or portal hypertension. The benign liver disease included liver cirrhosis and chronic hepatitis. None of 104 healthy control subjects were positive for the biomarkers of hepatitis viruses A, B, C, D, and E; HIV antibodies; or had liver; gallbladder; or kidney disease.

All blood samples were collected from subjects before treatment. The local Ethics Committees approved this study.

2.1. Methods

Peripheral blood samples were extracted from cases and controls prior to treatment. Fresh serum samples were shipped to clot for a period of thirty minutes at 37 °C. Samples were then separated by 3000 RPM centrifugal 10 min. The supernatant sera were collected and stored at −20 °C until testing. Although, AFU activity was assayed within 30 days after collection [12]. The serum AFU activities were assayed using a biochemistry analyzer. The serum levels of AFP were assayed by an electrochemiluminescence analyzer Eleusis 2010 with supporting reagents (Roche, German). The concentrations of TK1 were measured by using a commercial Kit based on an enhanced chemiluminescent (ECL) dot blot assay in accordance with instructions by the manufacturer (SSTK Ltd., Shenzhen, China). Normal reference values of AFU, AFP and TK1 were <40.0 U/L, <10.0 μg/L and <2.0 pmol/L, respectively.

2.2. Statistical analysis

Statistical analysis was performed using the SPSS 22.0 software package. Markers concentration distribution of patients in HCC, benign liver disorder and normal control groups were carried out by means of Kolmogorov–Sminov test, calculating the tumor marker concentration and Median (P_{25} and P_{75}).

Kruskal–Wallis and Chi-square tests were conducted for a comparison of tumor marker concentration levels among the different groups of subjects included. Comparison of continuous variables between the two groups was performed using the Mann–Whitney U-test. The receiver operating characteristics (ROC) curves, which correlated true-positive and false-positive rates (sensitivity and 1-specificity), were displayed. The areas under the ROC curve (AUC) were calculated for each biomarker as well. The statistical significance of differences after logistic regression between the two AUCs was also determined. Statistical analysis was conducted by logistic regression, analyzing diagnosis value of single and combined detection of the three tested tumor markers for HCC. The ROC curve was displayed with SPSS 22.0. In all tests, statistical significance was considered at P value less than 0.05.

3. Results

3.1. Study population

The present study was performed on a total of 225 patients with different liver diseases and 104 healthy subjects. Table 1 shows the major attributes of the subjects enrolled.

3.2. Tumor markers Kolmogorov–Sminov test

Statistical distribution of the three tested tumor markers in every group was Kolmogorov–Sminov test with $Z = 0.156–0.517$, $P < 0.001$, which was non-normally distributed, and non-parametric tests were used in the following statistical analysis.

3.3. Tumor marker concentrations in cases and controls

The concentrations of AFU, AFP and TK1 were statistically different among the three groups ($\chi^2 = 70.311–104.425$, all $P < 0.001$). AFP and TK1 levels were higher in HCC group than those in benign liver disorder group with the exception of AFU; AFU

Table 1
Clinical parameters of HCC group, benign liver disease group and normal control group [case (%)].

Items	HCC group ($n = 116$)	Benign disease group ($n = 109$)	Normal group (104)	F/χ^2	P value
Mean age (year)	57.8 ± 11.3	54.1 ± 11.9	56.9 ± 16.0	2.431	0.090
Gender				0.173	0.917
Male	75 (63.0)	72 (67.9)	70 (67.3)		
Female	41 (37.0)	37 (32.1)	34 (32.7)		
Etiology				0.551	0.458*
HBV	90 (77.6)	83 (76.1)			
HCV	18 (15.5)	21 (19.3)			
Others#	8 (6.9)	5 (4.6)			

* HCV versus HBV and others.
\# Including HAV, HDV, HEV and HIV.