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Detection of human liver-specific F antigen in serum and its preliminary application *

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INTRODUCTION

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity are usually used as biochemical criteria for the evaluation of liver lesion. These enzymes will release into the blood when the integrity of hepatocyte is compromised. Although the aminotransferase has been used as a criterion of liver function for decades, it can not show the accurate hepatohistological characteristics when it is abnormal in patients with chronic liver disease[1].

Human liver-specific F antigen (F-Ag), a M_r 44 000 cytosolic protein of unknown function, is found predominantly in the liver. Previous studies using relatively insensitive assays have shown that the concentration of F-Ag in the serum rises when the liver is severely damaged[2]. The sensitive radi oimmunoassay has enabled measurements in serum from healthy humans and patients with mild hepatic damage. The results suggest that F-Ag may be a sensitive and specific marker of hepatocellular damage[1]. To avoid the effects of rad ioactive isotope, Biotin-Avidin ELISA (BAEILSA) was established to detect F-Ag, and its clinical value as guidelines of liver function was discussed and compa red with that of AST and α-AFP.

MATERIALS AND METHODS

Materials

Subjects and serum samples The vein blood samples of 25 healthy subjects, 54 patients with various liver diseases and 10 patients with non-liver diseases were provided by Department of Gastroenterology of the First and Second Colleges of Clinical Medicine affiliated to Chongqing Medical University, and were centrifuged at 2 000r/min and stored at -20℃.

Reagents Standard F-Ag antibody (guinea pig against human F-Ag antiserum) purchased from DAKO (Denmark) and purified human F-Ag was used as the standard. F-Ag antibody (guinea pig against human F-Ag antiserum) was prepared by our department(1:32). D-biotin-N-hydroxysuccinimi-dyl esters (BNHS) and avidin-HRP (A-HRP) were provided by Shanghai Biochemistry Institute and Chemistry Department of Shanghai Medical University respectively.

Methods

Preparation of biotin-IgG (B-IgG) Oneg/L BNHS (resolv ed in N, N-dimethyl formamide) and purified anti-F-Ag IgG (v/v = 5:1) were mixed up quickly and placed for 4 h at room temperature. The mixture was dialysed to remove free biotin mixed with distilled glycerol (v/v = 1:01) and then stored at -20℃.

BA-ELISA Coating 96-well plate was coated with anti-F-Ag IgG (5 mg/L, 200 μL/well) diluted with 60 mm ol/L-$NaHCO_3$ (pH 9.6) and placed overnight at 4℃ or for 2 h at 37℃, blocked with buffer A (10 mmol/L PB, 150 mmol/L NaCl, 1.5% BSA, pH 7.6) for 30 min(200 μL/well). Standard F-Ag solution and serum samples were put into 96-well plate (100 μL/well) for 60 min . B-IgG and A-HRP diluted (1:200) with buffer B (10 mmol/L PB, 150 mmol/ L NaCl, 1% BSA, 0.05% Tween-20) were put into the plate again (100 μL/well). The substrate of HRP was 0-phenylenediamine (OPD) and H_2O_2 (100 μL/well). The reaction was stopped by 2 mol/ L H_2SO_4 (50 μL/well). The OD value of each well in the plate was detected under 492 nm wavelength. Concentration of F-Ag in serum was m easured in triplicate.

Detection of α-AFP and AST ELISA (normal value <20 μg/L) and routine assay of AST (normal value 5U/ L - 35U/L) were used.

Statistical method *t* test and correlative analysis

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were made by computer.

RESULTS

BA-ELISA

The minimum detection limit of BA-ELISA was 0.01U/mL (1U = 1.0 μg F-Ag). The linear range of the standard curve was 0.01U/mL-50U/mL. The intra-and inter-assay coefficient of variation (CV) was 6.2% and 7.7% respectively. Recovery rate was 95.9%-105.2% when the known amount of F-Ag was put into the serum of normal subjects. All serum samples were detected with BA-ELISA, and the results were shown in the follow ing Table 1. The serum F-Ag concentration of 25 healthy subjects was 0.08 U/mL ± 0.03 U/mL. The values under 0.08 U/mL ± 0.03 U/mL were regarded as negative. Compared with control group (healthy subjects), in crease was found in the serum F-Ag of all liver diseases with no change in non-liver diseases (P>0.05). AST activity was 124.5 U/L ± 62 .5 U/L- in non-carcinomatous liver diseases (31 cases). No obvious correl ation was found between their AST activity and serum F-Ag concentration(n = 31, r = 0.13, P>0.05). AST activity was 91.5 U/L ± 47.8 U/L in acute hepatitis (8 cases). A close correlation was found bet ween their AST activity and serum F-Ag concentration (n = 8, r = 0.89, P<0.05). The concentration of α-AFP in hepatocellular carcin oma (23 cases) was 113.7 μg/L ± 50.2 μg/L . An obvious correlation was found between their α-AFP and serum F-Ag (n = 23, r = 0.84, P<0.05).

Table 1 The serum F-Ag concentration of liver diseases and non liver diseases

Groups	Total cases	Positive cases	Positive rate (%)	F-Ag ($\bar{x}\pm s$,U/mL)
Control	25	0	0.00	0.08±0.03
Acute hepatitis	8	7	87.5	9.43±4.02
Chronic hepatitis	10	8	80.0	6.72±3.17
Hepatocellular cancer	23	21	91.3	15.15±5.48
Liver cirrhosis	8	6	75.0	8.31±3.59
Alcoholic liver diseases	5	4	80.0	5.28±2.64
Non-liver diseases	10	1	10.0	0.11±0.04

^aP<0.05; ^bP<0.01.

DISCUSSION

Making use of high affinity between biotin and avidin (K_d = 10^{-15} mol/L), we coupled biotin with anti-F-Ag IgG and developed BA-ELISA for detecting F-Ag of human serum which is highly sensitive, stable (recovery rate 95.9%-105.2%), easy and safe with a wide range (0.01 U/mL-50 U/mL). It is advisable for clinical use.

Positive serum F-Ag was found in 46 out of 54 cases of liver disease (its average level was 13.5 U/mL and its positive rate was 85.2%), while positive serum F-Ag was found in only 1 out of 10 cases of non-liver diseases. It indi cates that F-Ag is rather specific and has a high detectable rate for liver disease. Compared with present criteria for the evaluation of liver function, serum F-Ag level is correlated with AST activity in acute hepatitis patients, and is not correlated with AST activity in patients with chronic hepatitis, liver cirrhosis and cancer. It is suggested that F-Ag is of great practical value to the diagnosis, treatment and prognosis of liver diseases, and is a more sensitive and specific marker of liver damage and pathological features as compared with the routine criteria of liver function.

The detectable rate of serum F-Ag was 91.3% in 23 cases of liver cancer. The level of serum F-Ag was higher (the mean level was 14.3 U/mL) than that of other liver diseases. Serum F-Ag was negative in 4 cases of non-liver tumors out of 10 cases of non-liver disease, which was consistent with that of stomach cancer reported by Grewal[3]. It is suggested that serum F-Ag is a specific marker of liver tumor and has a high detectable rate. The level of F-Ag and α-AFP has a positive correlation. Therefore, detection of serum F-Ag is helpful for the correct diagnosis of liver diseases.

Further research is to be made in the function of F-Ag in order to find out the direct evidences and theoretical bases of F-Ag as a marker of liver damage.

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