---Short Communication---

DETECTION OF SERUM ALBUMIN RECEPTOR IN HEPATITIS B VIRUS CARRIERS BY ENZYME-LINKED IMMUNOSORBENT ASSAY

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Summary

The relationship between glutaraldehyde-treated polymerized human serum albumin (pHSA) and HBe antigen (HBeAg)-positive serum was examined by the use of a new enzyme-linked immunosorbent assay (ELISA). The author succeeded in measuring the pHSA binding activity (pHSA-BA) of HB surface antigen (HBsAg) particles in the present ELISA method using horseradish peroxidase-labelled pHSA after fixation of HBsAg on an anti-HBs-coated well of polystyrene microplates. In HBeAg-positive group, the pHSA-BA of sera of 40 asymptomatic carriers and 2 chronic persistent hepatitis (CPH) patients were higher than those of 8 chronic active hepatitis (CAH) (p<0.01) and 8 liver cirrhosis sera (p<0.05). On the contrary, in the anti-HBe-positive group the pHSA-BA of 17 asymptomatic carriers and 3 CPH sera were lower than those of 8 CAH (p<0.005) and 10 liver cirrhosis patient sera (p<0.005). In the both-negative group the pHSA-BA of 8 asymptomatic carrier and 3 CPH sera were also lower than that of 8 CAH (p<0.05). In acute exacerbation of HBsAg-positive CAH the pHSA-BA elevated one to two months before the peak of S-GPT level, being correlated with the DNA-polymerase activity. Because of its apparent reproducibility, it is concluded that low cost and some advantages may have clinical utility in the same setting as the HBeAg is now used.

Key Words: hepatitis B antigens, albumin receptor, virus receptor, HBeAg, ELISA.

Introduction

The diagnosis and management of HB surface antigen (HBsAg)-positive asymptomatic carriers with HBe antigen (HBeAg) are very important for prophylaxis of maternal transmission\(^1\) and horizontal infection\(^2\) by hepatitis B virus (HBV). It has been confirmed by numerous laboratories\(^3\)\(^{-9}\) by using hemagglutination, radioimmunoassay or immunofluorescence that polymerized human serum albumin (pHSA) treated with glutaraldehyde has an important role as a binding receptor for HBV. Recently detection of HBeAg-positive serum by radioimmunoassay and the pHSA-binding activity (pHSA-BA) is used for diagnosis of HBV-infectious markers in pregnant patients by using their serum\(^8\).

Based on these reports the author developed a new enzyme-linked immunosorbent assay (ELISA) method for detection of the pHSA-BA instead of the conventional pHSA-BA assay. With this the serum HBsAg is fixed on the anti-
HBs-coated well of microplates and the pHSA-BA of the patient serum can be easily measured with high sensitivity. The method and the clinical significance of this assay method are reported.

Materials and Method

Serum samples were obtained from 10 HBsAg-negative healthy persons, 65 HBsAg-positive asymptomatic carriers, 58 HBsAg-positive and 30 HBsAg-negative patients with chronic liver diseases (CLD). Eight patients were histologically diagnosed as chronic persistent hepatitis (CPH) type B, 24 patients as chronic active hepatitis (CAH) type B, 30 patients as CAH type non-B and 26 patients as liver cirrhosis type B (according to the International classification10). HBsAg-positive asymptomatic carrier and patient sera were divided into 3 groups, a) HBeAg-positive, b) anti-HBe-positive and c) HBeAg- and anti-HBe-negative. S-GPT level and other liver function tests were measured in fresh sera by the routine methods.

For detection of HBsAg, anti-HBs, HBeAg and anti-HBe, the RIA method (Abbot Laboratories, USA) was used. The titer of HBsAg and HBeAg was expressed as cut off ratio of c.p.m. For assay of DNA-polymerase (DNA-Pase) activity Kaplan’s method was used. For polymerization of human serum albumin (pHSA), the method of Lenkei and Ghetie12 was used. For preparation of horseradish peroxidase-labelled pHSA (PO-pHSA), the author used the method according to Nakane and Kawaoi13. The absorbances of pooled PO-pHSA was 0.34 at 280 nm and 0.10 at 403 nm. The PO-pHSA was diluted 100 times with 2 w/v % bovine serum albumin, 0.5 w/v % Tween 20 and 0.04 M Tris-HCl buffer pH 7.4 (TWEEN-Tris), divided into 3 ml of lots, and stored at -70°C.

For ELISA for pHSA-BA against HBsAg-positive serum, the anti-HBs-coated well of microplates (Greiner Co. USA) prepared by the method according to Wolters et al.14,15 or the purchased HBsAg-ELISA plate (Hepanostika®, Organon, Oss, The Netherlands) were used. 0.09 ml of 0.5 w/v % Tween 20, 0.04 M Tris-HCl buffer pH 7.4 (TWEEN-Tris) was added to a anti-HBs-coated well of microplates, then 0.01 ml of serum sample was added and incubated for 2 hours at 37°C in a moist chamber. The well was aspirated and washed 3 times with TWEEN-Tris buffer. Then 0.1 ml of immediately thawed PO-pHSA was added to each well, incubated for 1 hour at 37°C in the moist chamber, and removed by aspiration. The well was afterwards washed 4 times with the TWEEN-Tris buffer. 0.1 ml of a freshly prepared solution of o-phenylene diamine (0.4 mg/ml) and urea peroxide (0.2 mg/ml) in a phosphate-citrate buffer of pH 5.0 was added to each well and incubated in the dark for 50 minutes at room temperature. The enzyme reaction was stopped by adding 0.05 ml of 4 N sulphuric acid. The brown colour of the product of the enzyme reaction was read by measurement of the absorbance at 492 nm of a microcuvette of a Photo-Elisa II type colorimeter (140 µl cell) (Organon, Oss, The Netherlands). The titer of the pHSA-BA is represented using optical density (OD) ratio, and calculated by:

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\text{Titer of pHSA-BA} = \frac{\text{OD of test sample} - \text{OD of blank II}}{\text{OD of blank I} - \text{OD of blank II}}
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Blank I was the average OD of 10 HBsAg-negative control sera, and blank II the OD of 0.1 ml of a solution containing o-phenylene diamine and urea peroxide as described above, mixed with 0.05 ml of 4 N sulphuric acid.

For confirmation of positive pHSA-BA, each of 3 HBeAg-positive sera showing high titer (cut off ratio 7.0 by RIA in undiluted serum) was serially diluted with BSA-TWEEN-Tris buffer (undiluted, 1:3, 1:9, 1:27, 1:81, 1:243,