DETECTION OF HBe ANTIGEN IN SERA FROM HBs ANTIGEN ASYMPTOMATIC CARRIER AND HEPATITIS PATIENTS USING POLYETHYLENE GLYCOL (PEG)

Takao TSUJI, M.D., Takeshi OKADA, M.D. and Hideo NAGASHIMA, M.D.
The First Department of Internal Medicine, Okayama University Medical School, 5-1, 2-chome, Shikata-cho, Okayama 700, Japan

Summary

Serum and plasma samples concentrated 8 to 10 times with polyethylene glycol (PEG) having a molecular weight of 6,000 were examined by micro-Ouchterlony (MO) analysis with a view to increasing the detection sensitivity for HBe antigen (HBeAg), one of the hepatitis B virus associated antigens, and HBe antibody (HBeAb). The subjects of this investigation consisted of 82 symptom-free HBsAg carriers and 59 patients with B hepatitis. HBeAg was detected in 22 (26.8%) and HBeAb in 43 (52.4%) of 82 asymptomatic HBsAg carriers, and 17 (20.7%) were negative for both HBeAg and HBeAb. The corresponding values for the liver disease patients were 7 (11.9%), 16 (22.1%) and 36 (61.0%). Histologically, the rate of detection for HBeAg was higher in the cases of a mild disturbance.

Key Words: HBe antigen, HB surface antigen, asymptomatic carrier, polyethylene glycol (PEG).

Introduction

HBe antigen (HBeAg) which was discovered by Magnus and Espmark in 1972 has recently been shown to be closely related to HB virus infection. There are reports that the majority of chronic hepatitis patients with HBeAg positive serum are intractable and are liable to progress to liver cirrhosis. However, the method which is employed in the detection of HBeAg is at best Magnus' micro-Ouchterlony (MO) analysis or large well detection method either of which is by no means sensitive enough.

This paper deals with the results of an investigation which was undertaken to establish an HBeAg detection technique of high sensitivity using polyethylene glycol (PEG) which had been used by Poison et al. in the preparation of γG-immunoglobulins. In this investigation patient serum was concentrated with PEG in order to increase the sensitivity of detection for HBeAg and HBe antibody (HBeAb) and simplify the technique.

Materials and Methods

Serum samples were obtained from 59 patients with B hepatitis in whom HBs antigen (HBsAg) and HBs antibody (HBsAb) were...
determined by reversed passive hemagglutination (RPHA)\textsuperscript{10} and passive hemagglutination (PHA)\textsuperscript{11}, respectively, and plasma samples from 82 HBsAg positive blood donors with normal serum transaminase levels. Histological diagnoses of B hepatitis consisted of 3 cases of minimal hepatitis, 1 case of acute hepatitis, 3 cases of chronic persistent hepatitis (CPH), 6 cases of chronic aggressive hepatitis 2A (CAH, moderate), 23 cases of CAH 2B (severe), 11 cases of chronic lobular hepatitis (CLH), 3 cases of liver cirrhosis, 9 cases of hepatocellular carcinoma (HCC), 59 cases all told. Chronic hepatitis was classified according to the International Group\textsuperscript{12} and the European Classification\textsuperscript{13}.

Serum and plasma samples were concentrated with 31.5% polyethylene glycol (PEG) having a molecular weight of 6,000, and HBeAg and HBeAb detections were performed by micro-Ouchterlony (MO) analysis\textsuperscript{2}.

Three ml of 0.02 M acetate buffer of pH 4.6 was added to 0.5 ml of serum to adjust its pH and 1 ml of acetate buffer of pH 4.6 containing 31.5% PEG was added to adjust the final concentration of PEG to 7% (w/v), as shown in Fig. 1. The two solutions were mixed at 20°C, allowed to stand for 30 minutes, and centrifuged at 3,000 rpm for 15 minutes. The sediment was dissolved in a very small quantity of 0.005 M phosphate buffer saline (PBS) of pH 7.2. After standing at 4°C for 24 hours the solution was concentrated and used as a sample. The gel for MO assay consisted of 0.9% agarose dissolved in 0.01 M tris-HCl buffer of pH 7.6 supplemented with 0.1 M NaCl and 0.001 M EDTA. To increase the transparency of the gel it was filtrated with filter paper in the boiled condition and spread over a glass plate (10×10 cm) to a thickness of 2.0 mm. Wells 2.5 mm in diameter were made 6.0 mm apart from center to center, and the gel was cut away all the way around the well periphery. As a result, the diffusion of samples and reagents was facilitated so that the detection sensitivity for HBeAg and HBeAb could be increased. The concentrated sample and reagent were placed in each well and incubated at 37°C for 1 hour. After standing at room temperature the sample was examined every day and a final judgment made on the four day of observation. In the detection of HBeAg and HBeAb two HBeAg reagents and two HBeAb reagents which were discovered in HBsAg asymptomatic carriers by Laurell's electrophoresis\textsuperscript{14} and MO analysis respectively at the First Department of Internal Medicine of Okayama University were used. Each reagent was prepared from 100 ml of plasma in crude form using PEG and concentrated 8 times by volume. All these reagents gave a positive reaction to the previously described immunofluorescent complement (IFC) method\textsuperscript{15} for HBc antibody (HBcAb). Finally, these reagents were fixed by Prof. Okochi (Kyushu University, Kyushu)\textsuperscript{16} and Prof. Mayumi (Jichi

---

Fig. 1. Method of concentration

Sample (Serum or plasma): 0.5 ml
+0.02 M Acetate buffer pH 4.6: 3.0 ml
1.0 ml of 31.5% solution of PEG (MW: 6,000) in 0.02 M acetate buffer pH 4.6
[Prepared to a final concentration of 7% PEG (W/V), and kept at 20°C, 30 minutes]
↓
Centrifuged at 3,000 r.p.m., 15 minutes.
↓
Precipitate was suspended in a little volume (0.1 ml) of PBS pH 7.2.
↓
Suspension was kept at 4°C for 24 hours.