ASIAN PACIFIC JOURNAL OF ALLERGY AND IMMUNOLOGY (2000) 18: 115-117

Comparison of the RPHA and EIA Techniques for the Detection of HBs Antigen among Pregnant Thai Women

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SUMMARY Five hundred serum samples obtained from pregnant women attending an antenatal clinic in Bangkok were tested for HBsAg by reverse passive hemagglutination assay (RPHA) and enzyme immunoassay (EIA). It was found that 21 (4.2%) and 28 (5.6%) of the sera were positive by RPHA and EIA, respectively. The sensitivity and specificity of the RPHA were 75% and 100%, respectively, when using EIA as the standard method. The RPHA positive predictive value was 100% and the negative predictive value was 98.5%. Accuracy was 98.6%. This study showed that the RPHA was simple and required inexpensive equipment, making it suitable for mass screening. However, the possibility of false negative readings due to low levels of HBsAg should be kept in mind, especially in the blood transfusion practice.

Hepatitis is an inflammatory condition of the liver often caused by viral infection. Hepatitis, acquired after parenteral exposure to infected blood or body fluids, or to needles or equipment contaminated with infected blood, is usually due to either hepatitis B virus (HBV) or hepatitis C virus (HCV). Healthy individuals with a negative history can be carriers of one or more hepatitis viruses. In Thailand, the hepatitis B carrier rate is high, about 5-10% of the total population.[1,2] The detection of hepatitis B surface antigen (HBsAg) is one of the serologic markers of the hepatitis B infection stage which enables identification of HBV infected patients and assists in the diagnosis and prognosis of the disease. Also, the antenatal detection of infected pregnant women should encourage neonatal immunization against HBV. Although radioimmunoassay (RIA) and enzyme immunoassay (EIA) have been used as the standard techniques, the rapid detection of HBsAg in whole blood within minutes is useful to immunization or mass screening programs. Several methods have been introduced, such as reverse passive hemagglutination assay (RPHA), latex agglutination and immunochromatography.[3-5] The purpose of this study is to compare the sensitivity and specificity of the RPHA with the EIA for the detection of HBsAg in sera of pregnant women who attended an antenatal clinic in Bangkok.

MATERIALS AND METHODS

Subjects

Five hundred sera obtained from non-related pregnant women whose ages ranged from 17 to 44 years, with a mean age of 31 years, who attended the antenatal clinic at Phativej Paholyothin Clinic, Bangkok were included. The pregnancies varied from first to fourth parity.

Methods

The sera were kept at -20°C until tested for HBsAg by RPHA

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and EIA techniques. Both tests were performed in parallel by two different medical technologists. Immuno-comb II (Orgenics, Israel) was performed when any discrepancy in results between RPHA and EIA occurred.

RPHA

The commercial RPHA kit (Serodia-HBs, Fujirebio, Inc., Japan) was used. Qualitative assay procedures without a micropipette were performed according to the manufacturer's instructions. The agglutination patterns were compared with those of the reagent control according to the manufacturer's criteria: negative (-), indeterminate (±), 1+, 2+ and 3+.

EIA

The commercial Auszyme monoclonal EIA diagnostic kit (Abbott Laboratories, U.S.A.) was used. Procedure A assay was performed according to the manufacturer's instructions. The presence or absence of HBsAg was determined by relating the absorbance of the unknown sample to the cut-off value. Specimens with absorbance values greater than or equal to the cut-off value established with the negative value were considered positive for HBsAg.

Statistical methods

The sensitivity, specificity, positive predictive value, negative predictive value and accuracy were calculated.[6]

RESULTS

Twenty-eight (5.6%) of the serum samples were positive for HBsAg by EIA. Among these, 21 (4.2%) were positive by RPHA. Of the seven EIA positive samples not detected by RPHA, three were low positive, three were medium positive and one was a highly positive sample. Six out of these seven sera with negative results by RPHA were tested and identified as positive by Immuno-comb II.

Table 1 Comparison of the RPHA technique (RPHA) and the enzyme immunoassay (EIA) for the detection of HBsAg in 500 serum samples of pregnant women.

RPHA	EIA		Total
	Positive	Negative	
Positive	21	0	21
Negative	7*	472	479
Total	28	472	500

Sensitivity: 75%; specificity: 100%; positive predictive value: 100%; negative predictive value: 98.5%; accuracy: 98.6%.
*Six out of seven sera were positive for HBsAg by Immuno-comb II and one sera was not tested.

The sensitivity, specificity, positive predictive value, negative predictive value and accuracy of the RPHA were compared to those of the EIA (Table 1). It was found that RPHA gave a sensitivity of 75% while the specificity rate was 100%. The positive and the negative predictive values and the accuracy were 100%, 98.5% and 98.6%, respectively.

DISCUSSION

The RPHA commercial kit for HBsAg screening is commonly used in blood banks and clinical laboratories in Thailand. Although the EIA and radioimmunoassay are considered the most sensitive tests with specificity, they require special equipment, technical skill, and are time-consuming. RPHA has the advantages of being neither instrumentation-dependent nor labor-intensive and of being simple to perform. In this study, we compare the results obtained from the RPHA with the EIA, third generation Quantum immunoassay for the sera of pregnant women. It appeared that the RPHA offered a sensitivity of 75% and a specificity of 100%, confirming other findings regarding the RPHA.[3] Furthermore, the prevalence of HBsAg in pregnant women is 4.2% and 5.6% by RPHA and EIA, which is similar to previous studies and other studies in the Thai population. Due to the high prevalence of the carrier rate, about 5-10% in the total population, proper prophylactic intervention would be beneficial in the management of postexposure or vertical transmission.[1,2] Additionally, the RPHA failed to detect the presence of this antigen, especially in low or medium positive samples, which were shown positive by the EIA and the Immuno-comb II. This may be due to the lower limit of detection of each test. The lower limit of HBsAg detection by EIA, Immuno-comb II and RPHA are 0.3-0.7 ng/ml, 0.5 ng/ml and 20 ng/ml, respectively.[4,7]

In conclusion, our findings indicate that the RPHA may be used as a screening test because of its simplicity. However, the possibility of false negative readings due to low levels of HBsAg should be kept in mind, especially in blood transfusion practice.

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