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SHORT COMMUNICATION

Reversed Passive Hemagglutination Test Fails to Detect HBsAg in a Number of HBeAg Positive Sera

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SUMMARY **In endemic areas of hepatitis B virus (HBV) infection, perinatal transmission from asymptomatic HBsAg carrier mothers to infants plays a major role in the transmission of HBV. HBeAg indicates a high level of viral replication and infectivity. Most of the infants born to HBeAg positive mothers become carriers. Prenatal screening of HBsAg would identify infected mothers and thus allow preventive administration of immunoglobulin and immunization to the newborns. Reversed passive hemagglutination assay (RPHA) is commonly used in Thailand for HBsAg screening. However this method has low sensitivity and gives false negative results. Therefore, infants born to HBsAg false negative mothers would not receive proper immunization. This study reveals the rate of false negative results for HBsAg by RPHA in high infectivity sera. Of 985 sera which were HBsAg positive by ELISA, 70 (7.1%) were negative for HBsAg by RPHA. Of these 70 false negative sera, 7 (10%) were HBeAg positive. Our results indicate that RPHA is a less sensitive method for detection of HBsAg than ELISA. RPHA can give false negative results even in sera with high HBV infectivity. Therefore, RPHA should be replaced by EIA for prenatal HBsAg screening or any other screening for HBV infection whenever possible.**

Several methods can be used for detection of hepatitis B surface antigen (HBsAg) in serum. Reversed passive hemagglutination assay (RPHA) is a simple and inexpensive method suitable for mass screening which has been routinely used in Thailand for HBsAg screening of pregnant women. Prenatal HBsAg screening identifies infected mothers and thus allows preventive administration of hepatitis B immunoglobulin (HBIG) and immunization to their newborns.[1] However, many studies showed that RPHA had a low sensitivity and gave a large number of false negative results,[2-4] while enzyme immunoassay (EIA) was found to be the most sensitive technique. Since RPHA can give false negative results, infants born to mothers screened by this assay would not receive hepatitis B immune globulin (HBIG) prophylaxis treatment. These infants will have a high risk of becoming chronic carriers[5] and of developing chronic hepatitis, primary hepatocellular carcinoma or cirrhosis,[6] especially if their mothers were HBeAg positive.[7] In this study, the aim was to find the rate of false negative results for HBsAg detection by RPHA especially in highly infectious sera. The results from this study may be useful information for clinicians in choosing a prenatal HBsAg screening test.

MATERIALS AND METHODS

Subjects

The study population consisted of 40,723 healthy individuals who have been tested for HBsAg during July 2000 to January 2001 at the Workers' Health Screening Center of Ramathibodi Hospital.

HBsAg testing

Sera were tested for HBsAg with EIA technique using the Enzygnost HBsAg 5.0 (Dade Behring,

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U.S.A.). Positive samples from the Enzygnost were confirmed by microparticle enzyme immunoassay (MEIA) using the third generation microparticle EIA (AxSYM HBsAg V2 Abbott, U.S.A.). The quantitative signal of the MEIA was expressed as the S/N value as described by the manufacturer. Samples with S/N < 2.00 were regarded as negative while samples with S/N ≥ 2.00 were considered positive. Confirmed positive samples were tested by RPHA. The Serodia-HBs kit (Fujirebio, Japan) was used as RPHA test.

HBeAg testing

The EIA of Enzygnost HBe monoclonal kit (Dade Behring, U.S.A.) was used for HBeAg detection.

Statistical analysis

Statistical analysis of the association between quantitative HBsAg value at S/N > 100 and the positivity of RPHA was calculated by Chi-square. The relationship between quantitative HBsAg values and HBeAg was calculated by Fisher's Exact test.

RESULTS

Testing of HBsAg

Among the 40,723 healthy individuals tested, a total number of 985 HBsAg positive sera were found by EIA. All of the 985 sera were confirmed for HBsAg positivity by MEIA, and further tested for HBsAg by RPHA. Out of 985 samples, 915 (92.8%) were found to be positive by RPHA. The remaining 70 samples (7.1%), which were found negative by RPHA, were considered to be RPHA false negative. Therefore, the sensitivity of RPHA was 92.8% compared to the EIA.

Relationship of quantitative HBsAg values and HBsAg positivity by RPHA

The quantitative values of HBsAg by MEIA were compared with the HBsAg positivity by RPHA (Table 1). Among the 915 positive HBsAg sera by RPHA, 897 (98%) had a high load of HBsAg (S/N > 100) and 18 (2%) had an intermediate load of HBsAg (S/N 21-100). On the other hand, among the 70 sera which were HBsAg false negative by RPHA, the majority, 36 samples (51.4%) had intermediate S/N values (S/N 21-100). There were only 20 sera (28.6%) having a high load of HBsAg, (S/N >100). All 14 samples (20%) having low S/N values (S/N < 20) also belonged to the false negative RPHA group. These results clearly demonstrated the association of quantitative HBsAg values and the detective ability of RPHA ($p < 0.01$).

HBeAg in RPHA false negative samples

The 70 HBsAg false negative sera by PRHA were further tested for HBeAg. Seven sera (10%) were HBeAg positive. HBeAg results were analyzed with the quantitative HBsAg S/N values of these 70 sera (Table 2). Among the 7 HBeAg positive sera were 5 sera with a S/N value > 100, and 2 sera with S/N value of 98 and 55. These results demonstrated the association of quantitative HBsAg values and the detection of HBeAg ($p < 0.05$).

DISCUSSION

In this study, we reported the relationship between quantitative values of HBsAg by MEIA and qualitative screening results by RPHA. Most of the true positive HBsAg by RPHA sera had a high value of HBsAg by MEIA. In contrast, most of the false negative RPHA

Table 1 Comparison of the MEIA quantitative HBsAg S/N values and the qualitative HBsAg results by RPHA

MEIA (S/N)	RPHA	
	+	-
> 100	897	20[a]
20-100	18	36[a]
< 20	0	14[a]
Total	915	70

[a] p value < 0.01

Table 2 Comparison of the MEIA quantitative HBsAg S/N values and the qualitative HBeAg results in the HBsAg false negative RPHA sera

MEIA (S/N)	HBeAg	
	+	-
>100	5 (71.4%)	15 (23.8%)[a]
20-100	2 (28.6%)	34 (54.0%)[a]
<20	0 (0%)	14 (22.2%)[a]
Total	7	63

[a] p value < 0.05

sera had low or intermediate values of HBsAg by MEIA. Some sera with high levels of HBsAg were also become false negative, which was in accordance with another report from China.[8] There was a strong relationship between the quantitative load of HBsAg and the reaction of RPHA. This result, similar to other published studies,[2,9] confirmed that RPHA was particularly unsuccessful for the detection of HBsAg in sera with low or intermediate level of HBsAg.

To show that RPHA failed to detect HBsAg in high infectivity sera, the false negative sera by RPHA were tested for HBeAg. It was found that 10% of those false negative RPHA sera were actually HBeAg positive, which demonstrated that RPHA could not detect some highly infectious sera with hepatitis B virus.

Early detection of HBsAg in pregnant women can prevent infection in the newborns. Many studies have shown that combined prophylaxis with hepatitis B immunoglobulin and vaccine was the best method for prevention of perinatal transmission of HBV for high-risk infants.[10] Since HBeAg in mothers is a major risk factor determining perinatal transmission,[7] newborns of the false negative RPHA mothers with positive HBeAg would not have been given hepatitis B immunoglobulin and vaccine to prevent HBV infection. They would be exposed to a high risk to become chronic carriers, perpetuate the cycle, or acquire an associated malignancy.

Since HBsAg positive pregnant women are normally asymptomatic carriers, we can inferentially apply the results from this study for prenatal HBsAg screening in pregnant women. We conclude that EIA test is more accurate for prenatal HBsAg screening and, therefore, is more beneficial than RPHA. It should be used as the screening test of first choice for pregnant women if affordable. Such proper preventive measures for high-risk infants can be performed.

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ADDENDUM

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SPECIAL ARTICLE

Allergic Rhinitis and Its Impacts on Asthma: An Evidence-Based Treatment Strategy for Allergic Rhinitis

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Table 4 Strategy for the management of allergic rhinitis

Allergen avoidance is the first line of treatment

Mild intermittent disease	Moderate/severe intermittent disease	Mild persistent disease	Moderate/severe persistence disease	Management of conjunctivitis
Oral/intra-nasal H1 antihistamine	Oral/intra-nasal H1 antihistamines	Oral or intra-nasal H1 antihistamines	Intra-nasal glucocorticosteroids are the first line treatment (A stepwise approach is proposed)	Ocular H1 antihistamines
Intra-nasal decongestants (< 10 days)	Oral H1 antihistamines and decongestant	Oral H1 antihistamines and decongestant	If the nose is very blocked, a short course (1-2 weeks) of oral glucocorticosteroids or intra-nasal decongestants (<10 days)	Ocular chromones
Oral decongestants (usually not recommended in children)	Intra-nasal glucocorticosteroids (chromones)	Intra-nasal glucocorticosteroids (chromones)	Double the dose of intra-nasal corticosteroid if blockage is not better.	Saline
			Add H1 antihistamines if the major symptoms are sneezing, itching or rhinorrhea	Oral H1 antihistamines
			Add ipratropium bromide if the major symptoms is rhinorrhea	Ocular glucocorticosteroids only after the eye is properly examined
			Immunotherapy*	

*Immunotherapy may be used as the first line of treatment in moderate/severe persistent rhinitis

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