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Original Article

HBeAg testing is better than quantitative HBsAg assay as an alternative to HBV DNA assay among HBV-infected pregnant women

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Abstract

Introduction: Using tenofovir disoproxil fumarate (TDF) is recommended in the 3rd trimester for pregnant women with HBV DNA ≥ 200,000 IU/mL to prevent mother-to-child transmission (MTCT) of hepatitis B virus (HBV). However, HBV DNA quantification is unavailable in many resource-limited areas worldwide, hence prophylaxis is often missed. The aim of this study was to determine whether HBeAg or qHBsAg is a better alternative to HBV DNA testing in HBV-infected pregnant women.

Methodology: In this prospective cohort study, pregnant women with HBV infection were recruited in 3 hospitals from October 2019 to November 2020. Socio-demographic and clinical data were collected. Blood samples were taken for qHBsAg and HBV DNA testing. HBeAg results were collected from the medical records of the participants who visited a doctor during the study.

Results: 465 pregnant women met the study criteria. 41.9% were HBeAg positive, 33.3% had high qHBsAg levels (> 10^4 IU/mL), 38.3% had high HBV DNA levels (≥ 200,000 IU/mL). Pregnant women with high qHBsAg levels were 27 times more likely to have high HBV DNA levels (aOR = 27.0, 95% CI: 11.1-65.5, $p < 0.001$). Participants who were HBeAg positive were 57.5 times more likely to have high HBV DNA levels (aOR = 57.5, 95% CI: 23.0-140.0, $p < 0.001$). The sensitivity of qHBsAg and HBeAg was 80% and 94%, respectively; and specificity was 95% and 90%, respectively.

Conclusions: HBeAg testing should be considered over qHBsAg assay as an alternative to HBV DNA assay because of its technical simplicity, lower cost, and fewer missed treatments.

Key words: HBV; MTCT; pregnant; HBeAg; qHBsAg; HBVDNA.

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Introduction

Hepatitis B virus is a common chronic infection. Its complications include hepatitis, cirrhosis, and liver cancer, all of which create a great burden for the health system. More than 90% of chronic infection cases are due to mother-to-child transmission (MTCT) in endemic areas [1,2]. The prevalence of HBsAg in pregnant women ranges from 0.38-6.64% [3,4]. To prevent vertical transmission, pregnant women with hepatitis B virus (HBV) DNA level ≥ 200,000 IU/mL have been advised to receive tenofovir prophylaxis in the 3rd trimester.

HBV DNA assay is a gold standard for assessing viral replication but it is expensive and technically complex. Some studies have shown correlation between HBV DNA level and quantitative HBsAg level as well as a relationship between HBV DNA level and HBeAg status [3,5–7]. In 2017, The European Association for the Study of the Liver (EASL) recommended use of antiviral prophylaxis in pregnant women with HBV DNA ≥ 200,000 IU/mL or qHBsAg levels > 4 $\log^{10}$ IU/mL [8]. In 2020, The World Health Organization (WHO) stated that HBeAg testing could be used as an alternative to HBV DNA testing if HBV DNA testing is not available and that pregnant women with HBeAg-positive should receive tenofovir disoproxil fumarate (TDF) to prevent MTCT of HBV [9].

In this study, we sought to determine whether qHBsAg or HBeAg testing could serve as an alternative to HBV DNA testing to determine treatment eligibility for tenofovir prophylaxis in pregnant women in a resource-limited setting.

Methodology

Study design, population, and period

We conducted the prospective cohort study in three hospitals in southern Vietnam including the Hospital for Tropical Diseases, Tu Du Hospital and Dong Thap

General Hospital from October 2019 to November 2020. HBsAg-positive pregnant women who were not on antiviral therapy for at least 1 year at recruitment time were chosen for the study. In the Hospital for Tropical Diseases, we enrolled women who were in their 25th ± 2 weeks of pregnancy. These women were advised to take 300 mg per day of TDF until at least 1 month after birth if their viral load was greater than 200,000 IU/mL. In Dong Thap General Hospital and Tu Du Hospital, we recruited pregnant women who were prior to labor. A total of 665 eligible pregnant women were enrolled. Socio-demographic characteristics and HBeAg testing results were also collected. 6 mL of blood were taken from each participant. These blood samples were centrifuged, and the serum was separated and stored at -70 °C to -20 °C. They were transported to the Pasteur Institute in Ho Chi Minh City once a week to perform qHBsAg and viral load testing. Whether their infants had received hepatitis B immune globulin (HBIG) or not depended on their parents' affordability. All infants were vaccinated against HBV according to the national expanded program on immunization. These infants were tested for HBsAg and antiHBs to evaluate MTCT when they were one year old.

Pregnant women with hepatitis B virus infection who had HBeAg, qHBsAg, and HBV DNA testing results were selected for this study.

Table 1. Some main characteristics among pregnant women with HBV infection (n = 465).

Characteristics	All pregnant women, n (%)
Recruitment time	
23 ± 2 weeks of pregnancy	222 (47.7)
Preparing for labor	243 (52.3)
Age group	
18-25	105 (22.6)
26-35	306 (65.8)
> 35	54 (11.6)
Number of children	
0	210 (45.2)
1	181 (38.9)
2	65 (14.0)
≥ 3	9 (1.9)
Antiviral therapy history	
No	411 (88.4)
Yes	44 (9.5)
Unknown	10 (2.15)
HBV DNA (IU/mL)	
≥ 200,000	178 (38.3)
< 200,000	287 (61.7)
HBeAg	
Positive	195 (41.9)
Negative	270 (58.1)
qHBsAg (IU/mL)	
$>10^4$	155 (33.3)
$\leq 10^4$	310 (66.7)

Serological examination

HBsAg quantification assay (Abbott Diagnostics, Sligo, Ireland) is a two-step immunoassay using chemiluminescent micro-particle technology chemiluminescent microparticle immuno assay (CMIA) with flexible testing process for the quantitative determination of HBsAg in human serum or plasma.

HBV DNA quantification is determined by real-time polymerase chain reaction (RT-PCR)-based Cobas® (Roche Molecular Systems, New Jersey, USA; Roche Diagnostics GmbH, Mannheim, Germany) using primers and probes targeting the highly conserved pre-core and core region. It consisted of two phases: HBV DNA extraction and DNA amplification by using HBV-specific primer and probes for internal HBV genotype A-G and probes for internal QS.

The HBeAg tests were collected from the medical records that the pregnant women had available when they visited a doctor while taking part in the study. They were carried out during the pregnancy and could be done by other health facilities that are not the study sites.

Ethical approval

The study was approved by the Institutional Review Board of the Pasteur Institute in Ho Chi Minh City (number: 25/GCN-PAS signed on 15th August 2018). Informed consent was obtained at the time of enrollment. All eligible participants were fully informed about the general information of the study, the purpose of the study, the purpose of the blood test, risks and benefits of the study, and subject's rights.

Statistical analysis

Epidata 3.1 was used for data entry (EpiData Association, Odense, Denmark) and Stata 14.2 (Stata Corp LLC, College Station, TX, USA) was used for analysis. Categorical variables were reported as frequencies and percentages. Logistic regression analysis was used to determine the relationship between a dependent variable and other independent variables. *p* values of less than 0.05 were considered statistically significant.

Results

Participant's demographic and virological characteristics

A total of 465 HBV-infected pregnant women were included for analysis in which 222 (47.7%) pregnant women were enrolled at the Hospital for Tropical Diseases at 25th ± 2 weeks of pregnancy and 243

Table 2. The sensitivity and specificity of HBeAg testing and qHBsAg testing compared with HBV DNA assay (n = 465).

Characteristics		qHBsAg (IU/mL)		HBeAg		Total
		> 10^4 (n)	≤ 10^4 (n)	Positive (n)	Negative (n)	n = 465
HBV DNA (IU/mL)	≥ 200,000	142	36	167	11	178
	< 200,000	13	274	28	259	287
Sensitivity of tests (%)		80%		94%		
Specificity of tests (%)		95%		90%		

(52.3%) pregnant women were enrolled at Tu Du Hospital and Dong Thap General Hospital at the time of preparing for labor. The mean age of the study participants was 29.3 years old (range:18-42). The majority of pregnant women in this study were 25 to 36 years old (65.8%). Pregnant women with no or 1 child accounted for a large proportion in the study, 45.2% and 38.9%, respectively. 88.4% of the participants had received no antiviral therapy and 9.5% had used antiviral drugs but discontinued at least a year before the enrollment time. The percentage of pregnant women who had HBV DNA ≥ 200,000 IU/mL, positive HBeAg and qHBsAg > 10^4 IU/mL were 38.3%, 41.9%, and 33.3%, respectively (Table 1).

The sensitivity and specificity of HBeAg testing and qHBsAg testing compared with HBV DNA assay

When we consider HBV DNA ≥ 200,000 (IU/mL) as the criteria for TDF prophylaxis in pregnant women as the outcome, the sensitivity of qHBsAg and HBeAg were 80% (142/178) and 94% (167/178), respectively; the specificity of qHBsAg and HBeAg were 95% (274/287) and 90% (259/287), respectively (Table 2).

Association between the qHBsAg level, HBeAg status and others factors with HBV DNA level

Using univariate logistic analysis, we found that HBeAg status, qHBsAg level, recruitment time and age group were significantly associated with the HBV DNA level. Meanwhile, in multivariable logistic analysis, only HBeAg status and qHBsAg level had significant association with HBV DNA levels. Pregnant women who had high qHBsAg levels were 27 times as likely to have high HBV DNA levels compared to those with low qHBsAg levels (aOR = 27.0, 95% CI: 11.1-65.5, $p < 0.001$). Pregnant women who were HBeAg positive had a 57.5 times higher risk to have high HBV DNA levels than those who were HBeAg negative (aOR = 57.5, 95% CI: 23.0-140.0, $p < 0.001$) (Table 3). Others factors such as recruitment time, age group, number of children or antiviral treatment history were not associated with the HBV DNA level.

Discussion

The aim of the study was to find a viable alternative to HBV DNA testing for determining treatment eligibility for tenofovir prophylaxis in pregnant women in the resource-limited facilities. Some previous studies had shown a positive correlation between HBV DNA and qHBsAg level, as well as HBV DNA level and HBeAg status in HBeAg positive patients [10,11]. There was no correlation between qHBsAg and HBV DNA in HBeAg negative patients [12]. Our study found that both HBeAg status and qHBsAg level were statistically significantly associated with HBV DNA. Pregnant women who were HBeAg positive had a 57.5

Table 3. The factors associated with HBV DNA level.

Characteristics		HBV DNA ≥ 200,000 IU/mL	Univariate		*p* value	Multivariable		*p* value
			OR	95% CI		*aOR**	95% CI	
HBeAg	Negative	11 (6.2)	1		< 0.001	1		
	Positive	167 (93.8)	140.4	68.1-289.7		57.5	23-140	< 0.001
qHBsAg (IU/mL)	≤ 10^4	36 (20.2)	1		< 0.001	1		
	> 10^4	142 (79.8)	83	42.7-161.8		27.03	11.1-65.5	< 0.001
Recruitment time	23± 2 weeks of pregnancy	125 (70.2)	1		< 0.001	1		
	Preparing for labor	53 (29.8)	0.2	0.1 - 0.3		0.63	0.3-1.4	0.283
Age group	18-25	54 (28.7)	1			1		
	26-35	115 (64.6)	0.6	0.5-0.7	< 0.001	1.81	0.7-4.4	0.189
	> 35	12 (6.7)	0.3	0.1-0.5	< 0.001	3.07	0.7-14.1	0.150
Number of children	0	89 (50.0)	1			1		
	1	64 (35.9)	0.7	0.5-1.1	0.156	0.5		
	2	12.36 (12.4)	0.7	0.4-1.2	0.222	1,1		
	≥ 3	3 (1.7)	0.7	0.2-2.8	0.592	7.0		
Treatment history	No	151 (84.8)	1			1		
	Yes	23 (12.9)	1.9	1-3.5	0.047	0.9		
	Unknown	4 (2.3)	1.1	0.3-4.1	0.833			

***** Adjusted for recruitment time, age.

times higher risk of having high HBV DNA levels than those who were HBeAg negative (aOR = 57.5, 95% CI: 23-140, $p < 0.001$). In comparison, the association between qHBsAg and HBV DNA levels among pregnant women was lower. Pregnant women who had high qHBsAg levels were 27 times as likely to have high HBV DNA levels compared to those had low qHBsAg levels (aOR = 27.0, 95% CI: 11.1-65.5, $p < 0.001$) (Table 3). These findings suggest that, in the same population, when pregnant women were HBeAg positive, they had a higher risk of having HBV DNA ≥ 200,000 IU/mL than when they had of qHBsAg $>10^4$ IU/mL.

Moreover, the HBeAg test was more sensitive than the qHBsAg test (0.94 vs 0.80) but its specificity was lower than qHBsAg (0.90 vs 0.95). This suggests that, when using HBeAg testing as an alternative to HBV DNA assay, the omission risk of antiviral prophylaxis was lower compared to when qHBsAg testing was used and the likelihood of antiviral overtreatment (false positive) was higher compared to when qHBsAg testing was used (Table 2). To prevent MTCT, antiviral overtreatment should be preferred rather than omission of antiviral prophylaxis. Furthermore, HBeAg testing was more common than qHBsAg testing because of its accessibility, technical simplicity and lower cost.

Based on the above analysis, we believe that HBeAg testing is the better choice compared to qHBsAg testing as an alternative to HBV DNA testing to determine treatment eligibility for tenofovir prophylaxis in pregnant women in a resource-limited setting.

The study had some limitations. HBeAg results were collected from patient records and thus came from a variety of different labs. However, in reality, HBeAg testing is a routine follow-up for chronic HBV infection that is available in any health facility.

Conclusions

HBeAg testing compared to qHBsAg testing is the better alternative to determine if TDF prophylaxis is needed for pregnant women when DNA testing is not available or affordable.

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References

1. Piratvisuth T (2013) Optimal management of HBV infection during pregnancy. Liver International 33: 188-194. doi: 10.1111/liv.12060.
2. Li Z, Hou X, Cao G (2015) Is mother-to-infant transmission the most important factor for persistent HBV infection? Emerg Microbes Infect 4: e30. doi: 10.1038/emi.2015.30.
3. Liu D, Liu Y, Ni J, Li H, Zeng L, Zhang C, Zhang L, Yu Q, Wu B, Zhang L (2022) Hepatitis B infection among pregnant women in China: a systematic review and meta-analysis. Front Public Health 10: 879289. doi: 10.3389/fpubh.2022.879289.
4. Walker TY, Smith EA, Fenlon N, Lazaroff JE, Dusek C, Fineis P, Crowley SA, Benson R, Veselsky SL, Murphy TV (2016) Characteristics of pregnant women with hepatitis B virus infection in 5 US public health jurisdictions, 2008-2012. Public Health Rep 131: 685-694. doi: 10.1177/0033354916663183.
5. Alghamdi A, Aref N, El-Hazmi M, Al-Hamoudi W, Alswat K, Helmy A, Sanai FM, Abdo AA (2013) Correlation between hepatitis B surface antigen titers and HBV DNA levels. Saudi J Gastroenterol 19: 252-257. doi: 10.4103/1319-3767.121035.
6. Teriaky A, Al-Judaibi B (2013) Correlation between HBsAg quantitation and HBV DNA in HBeAg-negative HBV/D patients. Saudi J Gastroenterol 19: 243-244. doi: 10.4103/1319-3767.121030.
7. Belopolskaya M, Avrutin V, Firsov S, Yakovlev A (2015) HBsAg level and hepatitis B viral load correlation with focus on pregnancy. Ann Gastroenterol 28: 379-384.
8. European Association for the Study of the Liver (2017) EASL 2017 clinical practice guidelines on the management of hepatitis B virus infection. J Hepatol 67: 370-398. doi: 10.1016/j.jhep.2017.03.021.
9. WHO (2020) Prevention of mother-to-child transmission of hepatitis B virus: guidelines on antiviral prophylaxis in pregnancy. https://www.who.int/publications-detail-redirect/978-92-4-000270-8. Accessed: 2 October 2022.
10. Fouad R, Musa S, Sabry D, Salama A, Alem SA, Atef M, Zayed N (2020) Analysis of clinical and virologic features in Hepatitis B e antigen (HbeAg)-negative and HbeAg-positive Egyptian chronic hepatitis B patients. Afr Health Sci 20: 649-655. doi: 10.4314/ahs.v20i2.13.
11. Sun K-X, Li J, Zhu F-C, Liu J-X, Li R-C, Zhai X-J, Li Y-P, Chang Z-J, Nie J-J, Zhuang H (2012) A predictive value of quantitative HBsAg for serum HBV DNA level among HBeAg-positive pregnant women. Vaccine 30: 5335-5340. doi: 10.1016/j.vaccine.2012.06.036.
12. Ganji A, Esmaeilzadeh A, Ghafarzadegan K, Helalat H, Rafatpanah H, Mokhtarifar A (2011) Correlation between HBsAg quantitative assay results and HBV DNA levels in chronic HBV. Hepat Mon 11: 342-345.

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