Original Article

Quantitative HBsAg and Qualitative HBeAg Predicts Intrauterine Placental Infection and Umbilical Blood Cord in Pregnant Women

Erry Gumilar Dachlan; M.D., Ph.D.[1], Cahyanti Nugraheni; M.D.[1], Alphania Rahniayu; M.D.[2], Muhammad Ilham Aldika Akbar; M.D.[1,3]

1 Department of Obstetrics and Gynecology, Faculty of Medicine Universitas Airlangga, Dr. Soetomo General Academic Hospital, Surabaya, Indonesia
2 Department of Pathology Anatomy, Faculty of Medicine Universitas Airlangga, Dr. Soetomo General Academic Hospital, Surabaya, Indonesia
3 Department of Obstetrics and Gynecology, Universitas Airlangga Hospital, Surabaya, Indonesia

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Abstract

Objective: To know the correlation between quantitative Hepatitis B surface Antigen (HbsAg) and maternal Hepatitis B Envelope Antigen (HbeAg) with hepatitis B intrauterine transmission via placental infection. Hepatitis B in pregnancy causes a mother to child transmission (MTCT) via transplacental route started with placental infection. HBV DNA viral load and HBeAg are the independent risk factors for MTCT, but it rarely available in developing country.

Materials and methods: A cross-sectional study in 33 pregnant women with HbsAg positive in 4 referral hospital in East Java, Indonesia. Quantitative HBS Ag and HBeAg) status were determined serologically from a peripheral venous blood sample. Placental Hepatitis B infection was detected by immunohistochemistry of HBsAg from placental tissues. The intrauterine transmission was diagnosed by positive HBsAg in cord blood sampling after deliveries.

Results: Serum quantitative HBsAg level has a good sensitivity and spesificity to predict placental infection (90% and 83%), with a cut off value of 3.14 Log10 IU/mL (AUC 0.87; 95% CI: 0.74-0.99). Quantitative HBsAg level also has a good sensitivity and spesificity to predict HBV transmission in umbilical blood cord (81.8% and 95.5%) with a cut off value of 3.62 $\log_{10}$ IU/ml (AUC: 0.925, 95% CI: 0.813-1; p = 0.000). Placental infection is significantly related with intrauterine transmission with OR 4.6 (95% CI 2.29-9.4; p = 0.002).

Conclusion: The study reveals that maternal serum quantitative HBsAg level can be used as an alternative test to substitute HBeAg or HBV DNA as a marker to predict the placental infection and intrauterine transmission, especially in low-middle income countries.

Keywords: Hepatitis B; Placental Infection; Hepatitis B Envelope Antigen (HbeAg); Intrauterine Transmission

Introduction

Hepatitis infection is a common infection with a wide range of clinical appearance from acute hepatitis to chronic hepatitis, cirrhosis and hepatocellular carcinoma (1). As a tropical and coastal country,

Correspondence:
Dr. Muhammad Ilham Aldika Akbar
Email: muhammad-i-a-a@fk.unair.ac.id

Indonesia is the second country in Asia with the highest prevalence of Hepatitis B Virus (HBV) infection. In Indonesia, we found that 25-50% of patients with chronic HBV infections are transmitted through a vertical route (mother to child transmission). The prevalence of pregnant women with chronic HBV infection in this country is estimated at around 3-8%, which will lead to an increased risk of vertical transmission (2). While the prevalence of pregnant women with positive HBsAg ranged from 3.4% to 19.5% (3). 10-30% of infants of mothers with positive HBsAg and HBeAg negative become chronic carriers, whereas the incidence of infants who become HBV carriers in mothers with HBsAg and HBeAg positive is 80-90% (4). Indonesia showed the prevalence of children < 4years with hepatitis B is 3%, and the effectiveness of hepatitis B (HB) vaccination for prevention of intrauterine infection is 70-90% (5,6).

HB intrauterine transmission, among others, occurs by mechanisms through HB infection of the placenta, damage to the placental barrier and via hematogenous (7). HB transmission occurs through transfering by cell-to-cell transcytosis of the placenta or placental leak due to preterm delivery (8). Other mechanisms occur through HB infection in maternal morphonuclear cells that can transmit to the baby's bloodstream (9). HB can infect placental cells as evidenced by HBsAg immunohistochemical examination of all maternal placental cells (10).

Increased HBsAg titers were found to be associated with an increased risk of placental HB infection (8). HB infection in trophoblasts close to the villi capillary is most associated with the occurrence of intrauterine HB infection (10). HBV can also infect blood cells such as peripheral polymorphonuclear cells (PMNs), these cells become reservoirs and extrahepatic virus replication sites. This cell can enter the fetal blood circulation causing the transmission of HB to the fetus (11).

Various risk factors increase the risk of transmission of hepatitis infection such as the number of HB DNA, HBsAg titers and HBeAg. HBeAg is an important component of HBV that able to pass through the placental barrier and is a marker of active viral replication. The HBeAg positive status indicates the active state of the virus and increases the risk of intrauterine transmission (4). HBeAg will induce intrauterine fetal T-cell tolerance resulting in T-Helper cell failure in recognizing HBsAg and HBeAg (12). Ninety percent of babies born from mothers with positive HBeAg will become chronic carriers, while only 5% in mothers with negative HBeAg (7,13). High levels of HBV DNA and the presence of HBeAg are associated with the failure of HBV immunization and HBIG administration in neonates. Soewignjo's research in Mataram found that infants born to mothers with HBsAg and HBeAg positive, had a 66% HBV infection rate, compared to only 33.4% in negative HBeAg (14).

Controlling the strategy of mother to child transmission of Hepatitis B infection during this period of active and passive immunization in infants soon after birth would be applied largely (15). The provision of immunoprophylaxis is not always effective, as many as 10% of neonates will experience a failure marked by detection of HBsAg in 6 months infant blood after administration of immunoprophylaxis (16). This failure is caused by the intrauterine transmission, one of them through the mechanism of placental infection. Intrauterine transmission of HB infection via the placenta can not be prevented by administering the hepatitis vaccine (8). Therefore, prevention of placental HB infection is the first step in preventing the transmission of intrauterine HB infection.

In a developing country like Indonesia, the HBV in pregnancy is found with the national program of universal screening of HBsAg. HbsAg test has an overall good sensitivity and specificity (100% & 99.7%). After HBsAg is found in pregnant women, only a few patients continue to be examined with HBeAg, as recommended (17). This is caused by the availability of resources, geographic diversity, health coverage problems in our country. HBeAg is important as a sign of an active viral replication state. The occurrence of HBeAg in HBsAg positive pregnant women increased the risk of vertical transmission from 10 to 90% (18). In Indonesia, quantitative HBsAg examination is rarely performed in the management of pregnant women with HBV, while it may have a role in predicting the vertical transmission risk (1,11). Many studies have shown that placental infection may be involved in the development of intrauterine transmission to the newborn (19,20). Intrauterine transmission via the placenta may become one important mechanism of vertical transmission of HBV, besides the common pathway of direct exposure on the delivery process. While the actual prevalence of HBV vertical transmission in Indonesia is still uncertain, an ongoing study reveals that approximately 150.000

pregnant mothers have the potential to transmit HBV to their babies (21). In this study, we want to determine the relationship between maternal serum quantitative HBsAg level and HBeAg status with placental tissue infection and intrauterine transmission (indicate by HBsAg status in umbilical cord blood after delivery).

Materials and methods

Study Population: This was an analytic observational study with a cross-sectional method. It was conducted on 33 pregnant women with positive HBsAg in the 3rd trimester who delivered in 4 referral hospital in East Java, Indonesia (Dr. Soetomo General Hospital, Soewandhie General Hospital Surabaya, Haji Surabaya Hospital and Sidoarjo General Hospital) from September to November 2016. The samples were taken consecutively in these hospitals, based on inclusion and exclusion criteria. Inclusion criteria include pregnant women with Hepatitis B infection (HBsAg positive), who never had an antiviral therapy before, and agree to participate in the study. Exclusion criteria were patients with other viral hepatitis infection or co-infected with Hepatitis B (HBV), or any liver disease. Diagnosis of HBV infection is based on the positive serum HBsAg examination, before or during pregnancy. The study was approved by the Ethical Committee of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia. All patients involved in the study have the written informed consent taken by the researcher.

Ethical Approval: All procedures in this study was approved by The Ethical Committee of Dr. Soetomo General Academic Hospital (Surabaya, Indonesia) in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Serological Examination: Both quantitative HBsAg and qualitative HBeAg were measured serologically on the peripheral venous blood samples taken before deliveries. HBsAg was measured using Enzyme-linked Immunoabsorbent Assay (ELISA) methods on venous blood samples serum. Quantitative HBsAg measurements use Chemiluminescence Microparticle Immunoassay (ARCHITECi1000SR; Abbott©, Abbott Park, Illinois, USA), whereas HBeAg measurements use Electrochemiluminescence immunoassay "ECLIA" with a Coba-E immunoassay analyzer Elecsys© HBeAg quant - Cobas®; Elecsys corporation 846 N Martway CT, Olathe, KS 66061, USA). Intrauterine transmission is defined as the detection of HBsAg in the serum umbilical cord. The blood sample was taken from the serum umbilical blood cord right after the delivery of the baby. HBsAg was examined using the same methods as previously mentioned.

Placental Tissues Examination: Placental infection is defined as the result of the immunohistochemical examination, showed a positive HBsAg in the placental tissues. Placental biopsies were taken immediately after deliveries, with a size of 2 cm x 2 cm x full thickness. The placental tissues than fixated with 10% neutral buffered formalin then embedded in paraffin, sectioned into 5 um thickness, and then stained with the ABC methods (Avidin-Biotin Complex). Immunohistochemistry (IHC) was performed using an antibody against HBsAg (Monoclonal antibody IgG1 mouse code-gen MBL2-Novusbio; NBP1-22568) with 1:50 dilution. The semi-quantitative analysis of the stained sections was done by light-microscopy (400 times magnification) according to the immunoreactive score (IRS), evaluated blindly by one pathologist. Positive immunohistochemistry was defined as an occurrence of HBs antigen-antibody complex (brown stained cells) which is distributed in cytoplasm and membrane cells. Positive control in this IHC reading was the IHC staining in HBsAg positive from liver tissues. And the negative control was the IHC staining in HBsAg negative from placental tissues. Placental cells infected was defined as > 10% placental cells with IHC positive stained of HBsAg.

Statistical Analysis: The baseline data of maternal characteristics were descriptively analyzed. The categorical variables were compared between groups with the Chi-square test/Fisher's exact test, depending on the test requirement. The Odd ratio, sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of the test were also further analyzed. The interval/ratio data were compared between the group using the T-independent or Mann Whitney test, based on the normality of the data. The Pearson's or Spearman's rho correlation test was performed to evaluate the correlation between two variables. The ROC Curve was performed to evaluate the cut off value and Area Under Curve (AUC) of the quantitative HBsAg level to predict placental infection and intrauterine transmission. Data is processed using SPSS® 25 computer program (IBM© SPSS® 25).

Results

***Maternal Characteristics**:* The average age ± SD of participants in this study was 31.42 ± 6.1 years old, with a range of 21- 41 years. The mean ± SD age in the HBeAg positive group was younger compared to the negative HBeAg (29.73 ± 5.3 vs 32.27 ± 6.3 years old). Most of the participants were multigravida (70%), with the negative HbeAg group has more multigravida compared to the positive group (78% vs 50%). The mean ± SD gestational age at delivery was 38.12 ± 1.2 weeks with a range of 35 to 41 weeks. Most of the patients delivered aterm, only 6% from total participants delivered preterm (< 37 weeks). The majority of patients were delivered vaginally (63.36%), with the negative HBeAg group was more prevalent (70% vs 50%). There were no significant differences in age, gravidity, gestational age, and labor methods between positive vs negative HBeAg groups (Table 1). Baby birth weights ± SD range from 2150 grams to 4100 grams with an average of 3047 ± 420.69 grams. In the positive HBeAg group, the mean baby birth weight was slightly heavier compared to HBeAg (3170 ± 553,15 vs 2986 ± 334,97 gram). The number of low birth weight baby between both groups were similiar (10% vs 9%). Only 3 babies born with low Apgar score (< 7) at 5 minutes (9%). There was no significant difference in baby birth weight and Apgar scores between the two groups (Table 1).

There was no significant difference in both groups in terms of hemoglobin and ALT level (Table 2). The serum quantitative HBsAg level is significantly higher in positive HBeAg compared to the negative group (median: 6684,76 vs 1317,94 IU / ml; $p < 0.001$). Also, the log10 Quantitative measurement of HBsAg show the same result (positive vs negative HBeAg (mean ± SD): 3.76 ± 0.28 vs 2.84 ± 0.49 IU/ml; $p < 0.001$) (Table 2).

Relationship between Quantitative HBsAg and HBeAg, Placental Infection, Intrauterine Transmission: In this study, we measure and evaluate the relationship between serum quantitative HBsAg level with HBeAg status, placental biopsies result, and HBsAg status in umbilical cord blood. Serum quantitative HBsAg level is significantly higher in HBeAg reactive women compared to non-reactive group (median [range]: 19.708 (66-87287) vs 838 (159-2754) IU/ml; $p = 0.001$) (Table 2). The serum quantitative $\log_{10}$ HBsAg level also found same result (3.88 (3.07-3.96) vs 2.86 (2.02-3.88); $p = 0.000$). Based on the Spearman rho test, the serum quantitative HBsAg level has a significantly strong correlation with HBeAg status ($r = 0.589$, $p = 0.000$).

Table 1: General Maternal Characteristic

Category	Pregnant woman with Hepatitis B Infection		Total (n = 33)	P-value
	Positive HBeAg (n = 10) n (%)	Negative HBeAg (n = 23) n (%)		
Maternal age (years)				
< 30	3 (13)	0	3 (9)	0.34
30-35	5 (22)	0	5 (15)	
> 35	15 (65)	10 (100)	25 (76)	
Maternal Age (years)	29.73 (22-36)	32.77 (21-41)	-	0.35
Gravidity				
Primiparous	5 (50)	5 (22)	10 (30)	0.20
Multigravida	5 (50)	18 (78)	23 (70)	
GA at labor (weeks)				
< 37	0	2 (9)	2 (6)	0.45
37-40	9 (90)	18 (78)	27 (82)	
> 40	1 (10)	3 (13)	4 (12)	
Labor Method				
Vaginal delivery	5 (50)	16 (70)	21 (64)	0.45
Cesarean section	5 (50)	7 (30)	12 (36)	
Baby Birth Weight (gram)				
< 2500	1 (10)	2 (9)	3 (9)	0.19
2500-3500	6 (60)	17 (74)	25 (76)	
> 3500	3 (30)	2 (9)	5 (15)	
Apgar Score (first 5 minutes)				
< 7	2 (20)	1 (4)	4 (9)	0.95
≥ 7	8 (80)	22 (94)	30 (91)	

*Chi-square test

Table 2: Laboratory Characteristic of the participants

Category	Pregnant woman with Hepatitis B Infection		P-value	
	Positive HBeAg (n = 10) Mean (Range)	Negative HBeAg (n = 23) Mean (Range)		
Maternal age (years)	29.73 (22-36)	32.77 (21-41)	0.35	
Hemoglobin (g/dl)	10.97 ± 0.89[c]	11.36 ± 0.8[c]	0.33	
ALT (IU/ml)	28.9 ± 6.04[c]	32.72 ± 15.9[c]	0.58	
Quantitative HBsAg	19.708 (66-87287)	838 (159-2754)	< 0.001[a]	
Log_{10} Quantitative HBsAg (IU/ml)	3.88 (3.07-3.96)	2.86 (2.02-3.88)	0.000[a]	
	Placental infection positive (n = 10)	**Placental infection negative (n = 23)**	**P-value**	**Odd Ratio**
HBeAg Positive	7 (70%)	3 (13.1%)	0.002[b]	15.56 (2.5-95.7)
HBeAg Negative	3 (30%)	20 (86.9%)		
Log_{10} Quantitative HBsAg (IU/ml)	3.64 ± 0.31[c]	2.89 ± 0.56[c]	0.001	
	Intrauterine transmission positive (n = 11)	**Intrauterine transmission negataive (n = 22)**	**P-value**	
HBeAg Positive (n = 10)	8 (72.7%)	2 (9.1%)	0.000[b]	
HBeAg Negative (n = 23)	3 (27.3%)	20 (90.9%)		
Log_{10} Quantitative HBsAg (IU/ml)	8075 (66-87287)	884 (159-2884)	0.003[a]	
	Intrauterine transmission positive (n = 11)	**Intrauterine transmission negative (n = 22)**	**P-value**	
Placental infection positive	6 (54.5%)	4 (18.2%)	0.049[b]	
Placental infection negative	5 (45.5%)	18 (81.8%)		

[a]Mann Whitney Test; [b]Chi Square test, [c]mean+SD

Ten of the total 33 placentas were infected with Hepatitis B (30%), based on the placental biopsies. The infected placenta showed a significantly higher level of serum quantitative HBsAg compared to the uninfected placenta (3.64 ± 0.31 vs 2.89 ± 0.56 log_{10} IU/ml; p = 0.001). The serum quantitative Log_{10} HBsAg and placental HBsAg showed a linear data (p = 0.369, p > 0.05) based on the linearity test. Spearman rho test was performed to see the correlation between log_{10} HBsAg and Placental HBsAg, and the result was a significant (p = 0.012, p < 0.05) but weak relationship (CC: 0.433). The ROC (Receiver Operating Characteristics) curve was performed in further analysis. A quantitative HBsAg cutoff point for placental infection was 3.14 Log_{10} IU / ml (1380.38 IU / ml) with 90% sensitivity and 83% specificity with AUC area 0.87 (95% CI: 0.74-0.99).

Serum quantitative HBsAg level is also significantly higher in umbilical cord HBsAg positive (intrauterine transmitted HBV) compared to the negative group (8075 (66-87287) vs 884 (159-2884); p = 0.003). Serum quantitative HBsAg level also showed a strong correlation with the positive umbilical cord HBsAg (r = 0.520; p = 0.002) (Table 2). From the ROC curve analysis, the study reveals that the quantitative HBsAg cutoff point for intrauterine transmission was 1172.89 IU/ml with 81.8% sensitivity and 72.7% specificity with the AUC area 0.818 (95% CI 0.63-1; p = 0.003) (Figure 1).

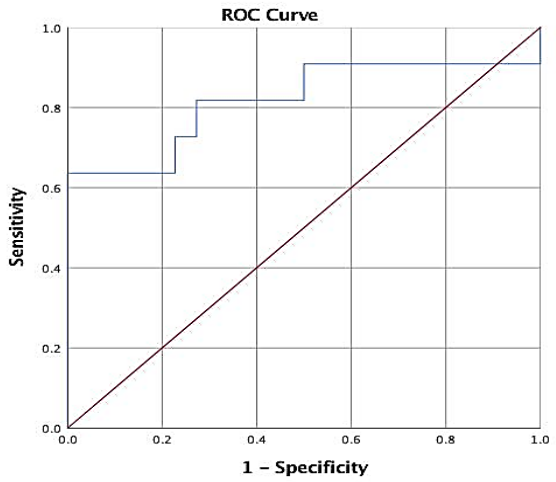

Figure 1: ROC curve of Quantitative HBsAg level (IU/ml) related to Intrauterine Transmission.

The quantitative Log10 HBsAg level also showed a higher level in positive umbilical cord HBsAg compared to negative group (3.86 (2.92-3.96) vs 2.83 (2.02-3.88); p = 0.000). With futher ROC Curve analysis, the quantitative HBsAg cut off value of 3.62 log_{10} IU/ml has very good predictive value (sensitivity: 81.8%; spesificity: 95.5%; AUC: 0.915; 95% CI: 0.813-1; p = 0.000) (Figure 2).

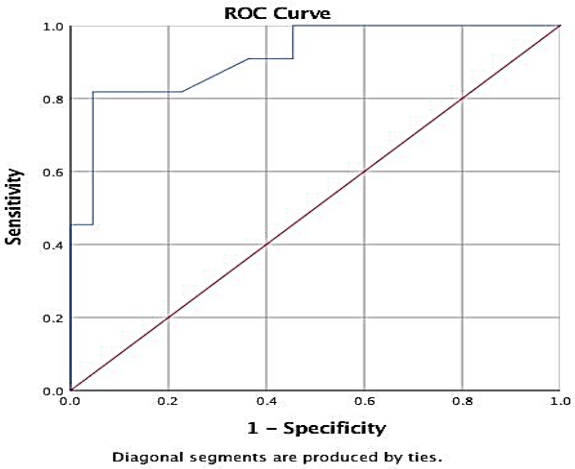

Figure 2: ROC curve of Quantitative HBsAg level (log10 IU/ml) related to Intrauterine Transmission.

Relationship Between Qualitative HBeAg with Placental Infections and Intrauterine Transmission: There were a total of 10 patients with positive HBeAg test (30.3%), from the total 33 participants. Seven of this positive group obtained a placental infection (70%). The negative HBeAg group shows a high percentage (86.9%) of negative placental infection, indicating a high specificity (Table 2). Based on the chi-square test, there is a significant difference between both groups (p = 0.002; OR: 15.56; 95% CI: 2.5-95.7). Further analysis indicate that HBeAg produce a good sensitivity (75%; 95% CI: 34.75-93.33%) and specificity (86.96%; 95% CI: 66.41-97.22%) to diagnose placental infection. The positive predictive value (PPV) and negative predictive value (NPV) of this test are 70% (95% CI: 42.97-87.85%) and 86.96% (95% CI: 71.85-94.57%), with the overall accuracy of 81.82% (95% CI: 64.54-93.02%).

From this study, we found that the intrauterine transmission rate was significantly differenced between positive and negative HBeAg pregnant women (p < 0.000) (Table 2). The HBeAg positive pregnant women have an Odds Ratio (OR) of 26.67 fold and relative risk (RR) 6,13 times to get an intrauterine transmission compared to negative HBeAg women. Sensitivity, specificity, PPV, and NPV of HBeAg test were as followed: 72.73% (39.03-93.98%), 90.91 (70.84-98.88%), 80% (50.41-94.03%), and 86.96% (71.57-94.64%). The association between HBeAg and Intrauterine transmission calculated with the Spearman Rho test was significantly strong (r_s = 0.652).

Relationship between Placental Infections and Intrauterine Transmission: The intrauterine transmission rate in this study was 33.3%, with 11 samples had a positive HBsAg in umbilical cord blood examination. The number of positive placental infection (diagnosed based on the findings of HbsAg in placental tissues) in this study was 10 samples (30.3%). The Chi-square test showed a significant difference between both groups. Patients with the confirmed intrauterine transmission, had a much higher prevalence of placental infection compared to negative intrauterine transmition groups (54.5% vs 18.2%, p = 0.049; OR: 5,4; 95% CI: 1.08-26,93). This indicating the possible association between intrauterine transmission and placental infection (Table 2).

Relationship between Type of Placental Infected Cell with Intrauterine Transmission: Apparently, this study found that viral intrauterine transmission was also associated with placental cell types (trophoblast cells, stroma, and capillary endothelial cells). All type of placental cells was infected with HBV, with a decreased percentage from endothelial cells, trophoblast cells, to the stromal cells (84.8% vs 33.3% vs 30.3%). In the intrauterine transmission positive group, the prevalence of infected cells were as follow: endothelial cells (100%), stromal cells (18%), and trophoblast cells (23%). Endothelial cell infection has a stronger association with intrauterine transmission compared to the trophoblast and stromal cells. Intrauterine transmission is associated with 100% endothelial cell infection, which is less found in stromal or trophoblast cells (Table 3). The odds ratio of intrauterine transmission in an endothelial capillary cell is 4.6 (95% CI: 2.29-9.4).

Discussion

This study reveals that the Quantitative HBsAg level has a strong correlation with HBeAg and HBsAg status in the umbilical cord blood, while it shows a weaker relationship with placental infection. In Indonesia and many developing countries, the HBsAg qualitative is often the only marker used to detect pregnant women with HBV because of the limited availability of the complete serum HBV markers test. Maternal HBV DNA (viral load), HBeAg, anti-HBeAb are ideally checked in the management of pregnant women with reactive HBsAg. Maternal serum HBV DNA (viral load) has been shown by many studies as a major risk factor for vertical transmission from mother to infants (11, 13, 16, 20).

Table 3: Correlation Between Type of Infected Cell with Intrauterine Transmission

Cell Type	Intrauterine Transmission		P-value	OR	95% CI
	Negative (n = 22) n (%)	Positive (n = 11) n (%)			
Trophoblast Cell					
Infected	6 (55)	5 (23)	0.049*	5.4	1.08-26.93
Non-infected	4 (45)	18 (77)			
Stroma					
Infected	6 (55)	4 (18)	0.049*	5.4	1.08-26.93
Non-infected	5 (45)	18 (82)			
Endothelial cell					
Infected	6 (55)	22 (100)	0.02*	4.6	2.29-9.4
Non-Infected	5 (45)	0			

But unfortunately in our country, this examination is unavailable in all hospitals related to the costly exam which is not covered by the national insurance, the wide-area or region to be covered (geographical difficulties), and often the unavailability of the reagents and the laboratory expertise. As a consequence, the HBeAg which is less expensive often chooses as a biomarker to predict vertical transmission. Although the availability of this test also limited in big referral hospitals or laboratory centers only. The HBsAg quantification may arise as a new alternative test in low-middle income countries since its available in a wider area and a lot cheaper than the HBV DNA or HBeAg cost.

In this study, maternal serum quantitative HBsAg level shows a strong correlation with the HBeAg status. HBeAg status indicated the activity or replication phase of the virus on the cells. HBeAg also has been shown by many studies as strong risk factors of maternal to child transmission of HBV, beside the HBV DNA. Quantitative HBsAg level is significantly higher in the HBeAg positive compared to the negative group (median: 6684 vs 1317 IU/ml; 3.88 vs 2.86 log10 IU/ml). This result is similar to another Indonesian study in another region by Fujiko et al (median HbsAg level in HBeAg positive vs negative group: 4.21 vs 2.91 $\log_{10}$ IU/ml) (2). Wen et al, in their study of 526 mother-infant pairs, also found a higher HBsAg level in HBeAg positive pregnant women (4.3 ± 0.8 vs 2.5 ± 1.1 log10 IU/ml; $P < 0.001$) (11). HBeAg is also correlated with maternal HBV DNA level, indicating the high risk of vertical transmission. Wen et al in their publication shows a higher viral load in HBeAg positive group compared to the negative one (7.3 ± 1.6 vs 2.9 ± 1.1 log10 IU/ml; $p < 0.001$). And the quantitative HBsAg level shows a strong positive correlation with maternal HBV DNA (viral load) ($r = 0.69$; $p < 0.001$) (11). In the latest study by Peng et al, quantitative HBsAg is correlated with maternal viral load ($r = 0.70$, $p < 0.001$), and highly predictive of HBV transmission in infants (22). In many developing countries, HBeAg and maternal HBV DNA (viral load) tests are rarely performed in the management of HBV in pregnancy. The high cost, unavailability of reagents and laboratory expertise limit these tests to be used in many low-middle income countries. Ideally, these test is used in predicting the risk of mother to child transmission, and to determine the need of antiviral therapy in pregnancy (16, 18, 23). With the finding that maternal serum quantitative HbsAg is well correlated with the HBeAg and HBV viral load, we may suggest this test can be used as a replacement/substituted of the HBeAg or HBV DNA examination in the management of pregnant women with HBV.

The maternal serum quantitative HBsAg level also predictive of the placental infection and intrauterine transmission in this study. Placental infection, nowadays, is considered as one of the important mechanisms of vertical transmission of HBV (13, 19, 20, 24). A higher level of quantitative HBsAg is found in the infected placenta, indicating a higher number of HBV in maternal serum which is "transfer" to the placental cells. The cut-off value of the quantitative HBsAg level at 1380.38 IU/mL has very good sensitivity and specificity to predict the risk of placental infection (AUC 0.87). Furthermore, the maternal serum quantitative HBsAg level also shows a strong correlation with the HBsAg status in the umbilical blood cord, which is associated with the intrauterine transmission process. Neonates with positive umbilical cord HBsAg tend to have a low response to vaccination resulting in vertical

transmission, caused by in-utero infection (14, 25). From this study, we found that serum HBsAg level > 1172.89 IU/ml has good sensitivity and specificity (81.8% and 72.7%) to predict intrauterine transmission (AUC 0.818). These findings are similar to Wen et al study, which found that quantitative HBsAg predicts infant infections as well as maternal viral load (11). Serum HBsAg and HBV DNA may reflect the overall activity of the virus, but with slightly different virology. Active replication of the HBV can be measured from the HBV DNA level. On the other hand, the HBsAg is reflecting viral particle which is produced not only from mRNA of active covalently closed circular DNA (cccDNA) but also from HBV DNA sequences (26). With this overall good predictive value of placental infection and intrauterine transmission, we suggest that maternal serum quantitative HBsAg may be used as an alternative of maternal viral load to predict the risk of intrauterine transmission, especially in the low-middle income countries with limited access to complete laboratory test.

The HBeAg level in this study is also correlated with placental infection and intrauterine transmission. HBeAG is a small antigen that can cross the placenta from the mother to fetus (6). The placental infection rate in HBeAg positive group is higher compared to the negative group ($p = 0.002$), with the OR 15.56 fold. Wei's study found similar results that placental infection rate from mother with a positive HBeAg was higher compared to the negative HBeAg, with lower Odds Ratios compared to our study (OR = 2.00; 95% CI 1.02-3.95) (8). In the placenta of pregnant women with positive HBeAg, 82% was infected with HBV indicating the strong association between HBeAg and placental infection (8,27). In a large study done in China involving 1133 HBsAg positive mothers, maternal HBeAg pos itive has been shown as a strong independent predictor for intrauterine transmission (OR: 2.56, 95% CI: 1.54-4.27) (28). HBeAg level has moderate sensitivity (> 70%) and good specificity (> 90%) to predict both placental infection and intrauterine transmission in our study. The NPV of HBeAg test to diagnose placental infection and intrauterine transmission is higher compared to the PPV. These findings indicate the importance of negative HBeAg status in predicting the low risk of placental infection and intrauterine transmission.

The placental biopsies in this study revealed that the HBV infected all placental cell types. The placental infection rate of HBV was 30%, and the rate was decreased toward the cell that more closed to the fetal side. The rate of infection in endothelial, stromal, and trophoblast cells are as followed: 84.8%, 30.3%, and 33.3%. This result is supported by the Xu study, which used immunohistochemistry for the detection of hepatitis B infection in the placenta of 158 pregnant women with positive HBsAg. The result shows that the placental infection happened in all cells type, but with a higher infection rate (58.8%) (20). Detection of the virus on the placenta using initiated hybridization DNA also found the virus in various cells in the placental layer with a decreased concentration from the maternal to the fetal direction (20). In another study, placental virus detection using DNA hybridization insitu found HBV in all placental tissue layers, with decreased concentration from maternal to fetal side (8). This placental layer to layer methods combined with bloodborne transmission will lead to cell-mediated infection (20, 29). This cellular transfer involves bonding between HBs antigen-antibodies complex with transmembrane protein Fcγ (especially FcγIII) found on the cell membrane of placental tissue (27, 30). All of these findings suggest that HBV transmission occurs via inter-layer cell transfer of infected placental tissue so that even without direct contact between maternal and fetal blood vessels (such as in the preterm labor or premature rupture of the membrane), the vertical transmission can still occur.

Transmission of HBV infection intrauterine is preceded by the occurrence of placental infection, which heading to the umbilical blood cord, and finally the fetal circulation (19, 20). The mechanism of this transmission varies, but it involves two important molecules: the HBV immune complex, and the Maternal Peripheral Blood Mononuclear Cell (PBMC) (20). In this study, the overall intrauterine transmission rate was found at about 32%. There is a significant correlation between intrauterine transmission with placenta infection, with an OR of 5.4. The association was more significant in infected capillary endothelial cells with OR 4.6. All samples with infected capillary endothelial cells have a positive HBsAg in umbilical blood cord (100%), indicating an intrauterine transmission. The HBsAg is a component of HBV with large particle (2.7 nm) which can not directly penetrate the placenta. But it can pass the placental barrier through cellular transfer

mechanism as previously explained. Intrauterine transmission is significantly associated with placental infection especially when infection occurs in capillary endothelial cells (10, 31, 32). There were 5 samples with positive placental Hepatitis B infection but no intrauterine transmission. Infection only limited to trophoblast and stromal cells without endothelial cells. In contrast with 4 samples without placental infection, which is found to have an intrauterine transmission. This is the point that intrauterine transmissions may also occur through other pathways other than transplacental "layer by layer methods", such as transmission via the maternal PBMC cell. The virus can infect PBMC cells and replicate therein, which then migrates to the blood circulation fetus (9, 11).

Conclusion

The study reveals that maternal serum quantitative HBsAg level can be used as an alternative test to substitute HBeAg or HBV DNA examination in the management of pregnant women with HBV, especially in limited resources hospitals. Maternal serum quantitative HBsAg level can also be used as a marker to predict the placental infection and intrauterine transmission. With this finding, we suggest to include the maternal serum quantitative HBsAg level in the protocol management of HBV in pregnancy, in a country with limited availability of complete HBV test. High serum quantitative HBsAg level may indicate a high risk of vertical transmission, which needs antiviral therapy in pregnancy. Although the actual cut off value of this marker to start the antiviral therapy need to be determined in the future study. And this study also found that the HBV can infect all placental cells, indicating the importance of placental infection in the mechanism of intrauterine transmission.

Conflict of Interests

Authors have no conflict of interests.

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