

**Original Article**

# Significance of HBsAg Expression in Placental Tissues with Serum positivity in Hepatitis B virus-infected Mother

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## ABSTRACT

**Introduction:** Hepatitis B virus infection in fetal and newborn may result from the intrauterine vertical transmission. The purpose of our study is to determine the association between HBsAg expression in the placental and HBsAg serum in pregnant women. **Methods:** Total 67 placental tissues and serum were obtained from HBV-infected mothers. This study was conducted in various hospitals in Makassar, Indonesia from December 2017 until August 2018. Maternal serum and placental tissues were tested by ELISA and Immunohistochemistry staining. **Results:** 67 samples from placental tissues and serum were categorized into two different levels of serum titer and staining positivity. Twenty nine samples (43.3%) were categorized in low titer of HBsAg positive serum and 38 samples (56.7%) in high titer of HBsAg positive serum. From the total of 67 placental samples, 43 (64.2%) samples had positive expression of HBsAg immunohistochemistry staining and 24 (34.8%) samples had negative expressions. **Conclusions:** There was a significant correlation between the expressions of HBsAg in the placental tissues and serum titers of HBV-infected mothers that is important in predicting the risk of transplacental transmission. The results of this study can be used as guidelines for the prevention and therapeutic approach of mother-to-child HBV infection.

## 1. INTRODUCTION

Hepatitis B virus (HBV) infection is a worldwide health problem that has been infected more than 2 billion people. Around 240 million of people were chronically infected by HBV, mostly from low-income countries. The World Health Organization (WHO) reports that about 650,000 people were died due to severe complications of cirrhosis and liver cancer.<sup>1,2</sup> The prevalence of this chronic infection was high in Southeast Asia, where about 70-90% of the population was infected under 40 years old.<sup>3</sup>

Transmission of Hepatitis B virus mainly through blood transmission, sexual intercourse, childbirth process, and perinatal vertical transmission from infected mother.<sup>4</sup> Vertical transmission of HBV in Asian countries mainly occur in pregnant mothers to their child. The prevalence of mother to child transmission of chronic HBV infection is 0.82%.<sup>5</sup>

One-third of chronic hepatitis B viral infections worldwide was mother to child vertical transmission. Although prophylactic efforts after exposure have been carried out to reduce the infection, it is found that 5–15% of infants still infected with HBV. Hepatitis B surface antigen (HBsAg) and hepatitis e antigen (HBeAg) in pregnant women with HBV DNA level of 106 copies/mL (>200,000 IU/mL) have the most significant risk of transmitting HBV to their babies.<sup>6</sup> In Indonesia, the risk of vertical transmission was high because the high numbers of mother were infected with hepatitis B virus. Trans-placental transmission (in utero) can occur in 3.7% of mothers with positive HBsAg, and 9.8% show positive HBeAg. Factors associated with intrauterine transmission are viral load, HBeAg, maternal HBsAg titer, and genotype of hepatitis B virus.<sup>7</sup>

Mother to child transmission of HBV can develop in many different ways including intrauterine transmission, childbirth transmission, postnatal care, and during breastfeeding period.<sup>8</sup> Several potential mechanisms for intrauterine transmission of HBV are leakage of the placental barrier, infection of placental, contagion from blood mononuclear cells cell and transmission from the germline pathway.<sup>9</sup>

## **2. METHODS**

Total 67 placental tissues and serum were obtained from HBV-infected mother. This study was conducted in various hospitals in Makassar, Indonesia from December 2017 until August 2018. Maternal serums were tested using ELISA kit (Bio-Rad, Marnes-la-Coquette, France) with a qualitative immunoassay principle method. The assays based on specific monoclonal antibody that will bind with HBsAg protein. The assay cut-off <0,080 was determined as negative, and a cut-off of >1.0 was determined as positive.<sup>10</sup> Placental tissue samples were cut including the fetal and maternal parts in minimum size of 2x2 cm before treated into paraffin blocks. Paraffin blocks were stained using immunohistochemistry staining. The immunoassay stains were performed with deparaffinization and rehydration procedures using serial concentration of ethanol 100%, 90%, 80%, and 70%. Citrate buffer was used for the antigen rehydration for 10 minutes at 103 ° C before incubated with 3% of hydrogen peroxide for about 10 minutes in room temperature for blocking any endogenous peroxidase. Tris buffer saline (TBS) was used for washing period before incubated in 10% BSA (bovine serum albumin) for 30 minutes in a humidified room at room temperature. Special part that had been restricted using PAP pen was filled with 5 µg/mL of primary

antibody, HBsAg (A10F1, CELL MARQUE) and incubated up to 2 hours at room temperature. Before incubated and conjugated with HRP-secondary antibody, the slides must be rinsed three times with TBS for three minutes. Slides were incubated and washed with diaminobenzidine (DAB) substrate, then counterstained with hematoxylin. Finally, slides were dehydrated and installed with mounting agent.<sup>11</sup>

Determination of HBsAg expression was confirmed when stained turn brown on membrane and cytoplasm of trophoblast cells using a light microscope with 400x objective magnification by two pathologists blinded to clinical information. Outcomes expressions were divided into two groups: negative expression if cytoplasm and cell membrane of the trophoblast were not stained, and positive expression if cytoplasm and cell membrane of the trophoblast were not stained.<sup>12</sup>

All analyses in this study were processed using SPSS 20 for Windows software. Each group will be summarized as mean±SD and compared using the Spearman correlation test and Mann-Whitney test. For  $p < 0,05$  all p values was considered statistically significant. This study has been approved by the Faculty of Medicine Hasanuddin University Ethical Committee with batch Number: 526/UN4.6.4.5.3.1/PP36/2020.

### 3. RESULTS

Table 1 described the distribution of HBV-infected mother based on age.

**Table 1. Age of HBV infected mother**

<b>Variable</b>	<b>Age (years)</b>
Mean	28
Median	28
Minimum	18
Maximum	39

Based on the table above, it was showed that total sample of HBV-infected mother was 67 samples with mean of age 28 years old. The youngest age was 18 years, and the oldest was 39 years.

HBV-infected mother with positive HBsAg serum were categorized based on the titer positivity as showed in table 2 based on ELISA results. There were 29 samples (43.3%) with low positivity and 38 samples (56.7%) were high positivity.

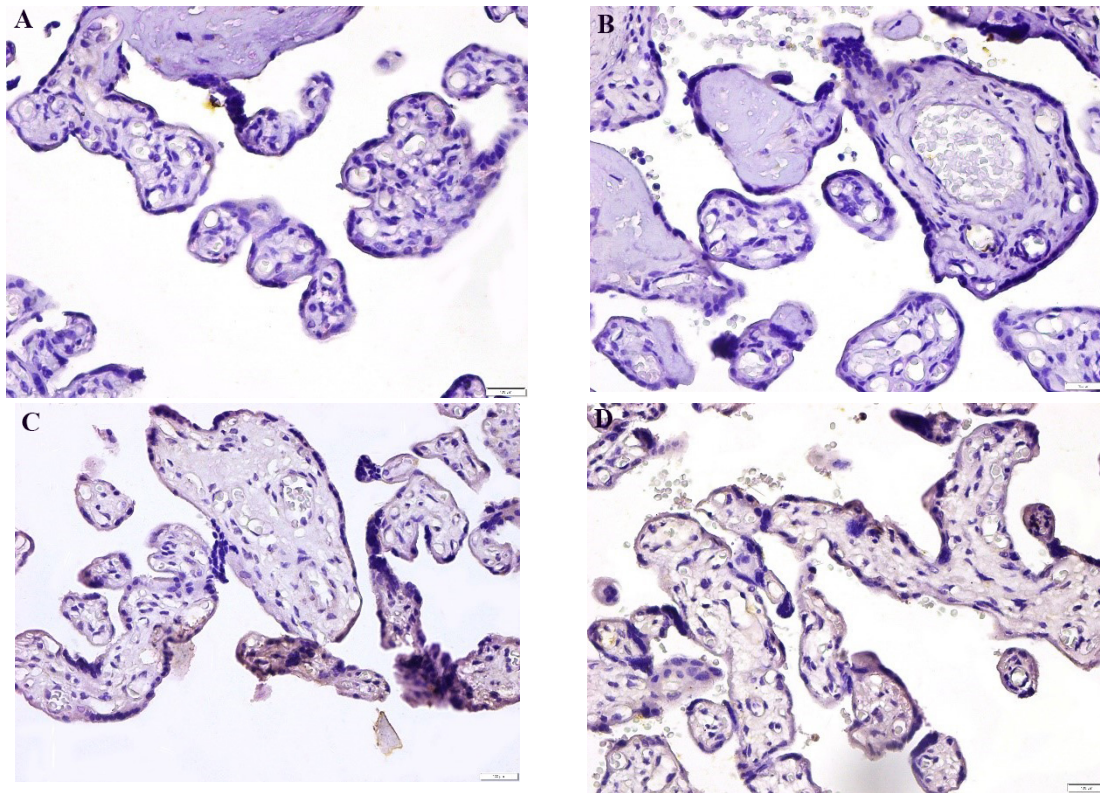
**Table 2. Titer positivity based on serological test (ELISA)**

<b>Titer Serum of HBsAg</b>	<b>n (%)</b>
Low	29 (43.3)
High	38 (56.7)

Results of immunohistochemical assay of 67 samples from placental tissues were described in table 3. There were 43 samples (64.2%) from total samples, showed positive expression of HbsAg staining on the placental cytoplasm and membranes of trophoblastic cells and 24 samples (35.8%) showed negative HBsAg expression. The placenta staining with HBsAg antibodies immunohistochemistry results can be seen in Figure 1.

**Table 3. Based on HBsAg Expression in Placental Tissue**

<b>HBsAg expression on Placental Tissue</b>	<b>n (%)</b>
Positive	43 (64.2)
Negative	24 (35.8)



**Figure 1.** Expression of HBsAg. A-B. Negative of HBsAg expression (40x magnification). C-D. Expression of HBsAg positive stained on the membrane and cytoplasm of trophoblast cells in the placental villi (40x magnification).

**Table 4.** Correlation of HbsAg expression in Placental tissue with HbsAg serum titers

		HbsAg serum titers			P-Value
		n	Median (Minimum-Maksimum)	Mean $\pm$ SD	
HbsAg expression in Placental tissue	Negative	24	0.05 (0.05-3674)	481.83 $\pm$ 1005.55	0.001*
	Positive	43	5048 (0.05-96360)	14074.79 $\pm$ 23401.54	
Total		67			

\*Mann-Whitney Test

Table 4 showed that there was a significant correlation between HBsAg expression on placental tissue with the level of HBsAg serum titer ( $p < 0.05$ ).

**Table 5.** Correlation analysis of serum HBsAg titer with HBsAg expression in the placenta

Titer HbsAg	$r = 0.734.$
	$p < 0.0001$
	$n = 67$

Table 5 shows the  $p$ -value  $< 0.0001$  with  $r$  value = 0.734, indicating a significant correlation between maternal blood HBsAg titer levels and the immunohistochemical expression of HBsAg in placental tissue with a strong correlation value.

#### 4. DISCUSSIONS

Hepatitis B is one of the global health problems that can cause several liver diseases like hepatitis, liver cirrhosis, and liver cancer. Hepatitis B virus is an infectious agent that can infect pregnant mothers and their child. The most common cause of hepatitis B infection in perinatal period is a transmission from mother to baby that occurs intrauterine or perinatally. Hepatitis B virus can penetrate the placental and cause infection. Pregnant women who are HBsAg(+)/HBeAg(+) have a high risk to transmit the virus to their babies (70-90%) compared to pregnant women who are HBsAg(+)/HBeAg(-).<sup>13</sup> According to HBsAg and HBeAg positivity levels, high HBsAg titers are a significant factor in of mother-to-child vertical transmission.<sup>14</sup>

In this study, the age of pregnant women detected with positive serum HBsAg

obtained a mean value of 28, median 28. It can be concluded that the sample group studied has a tendency to develop hepatitis B at a young age. WHO states that detecting serum HBsAg at a young age indicates that prophylactic immunization has not been entirely done. The earlier age of a person infected with the hepatitis B virus, the greater the chance of becoming a chronic infection.

From our result, there was a significant correlation between the levels of HBsAg serum titer with HBsAg expression in placental tissue. Expression of HBsAg immunostaining in the placenta, although not in the entire placenta, may indicate the risk of intrauterine infection. The literature states that serum HBsAg titers are positively correlated with HBsAg expression in the placenta and maternal serum HBV DNA. Previous study stated that the expression of HBsAg was found in the cytoplasm of trophoblast cells with a patchy pattern.<sup>15</sup> Moreover, our result shows a significant correlation between HBsAg serum titers (>0.05 IU/ml) and the immunohistochemical expression of HBsAg in the placenta cell. A previous study has showed that there was a mutual relationship between viral replication markers in the serum and the expression of HBsAg<sup>16</sup>

The strong association between placental HBsAg expression and maternal serum HBsAg suggests that HBsAg expression may be considered a histologic manifestation during the evolution of the HBV genome under host immune stress. This study revealed an immunohistochemical expression of HBsAg in the placenta with a patchy pattern in HBV-infected mother without distinguishing between acute and chronic phases, which was characterized by varying serum level. Trans-placental HBV transmission is a risk factor for residual. Mothers with HBV infection have a high risk of giving birth to babies infected with hepatitis B via the transplacental route, even though appropriate prophylactic immunization has been carried out.<sup>17</sup> In this study, there is a possibility of HBV vertical transmission through trans-placental route characterized by the expression of HBsAg on the membrane and cytoplasm of placental trophoblast cells. Therefore, it is necessary to pay attention to conducting early screening of pregnant women to detect and take effective action in preventing mother-to-child virus transmission.

## **5. CONCLUSION**

Significant correlation between HBsAg expression on the placental cell and serum positivity is important in predicting the risk of vertical transmission. Expression of HBsAg in the placenta tissues is an important feature that indicating the probability of virus infection. The presence of HBsAg expression, even in small amounts or patchy in the placenta of HBV-infected mother, can be used as a marker of trans-placental transmission of hepatitis B virus from mother to child where the mechanism of transmission is still unknown.

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**Conflict of Interest Statement:**

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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