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Asialoglycoprotein receptor expression in placenta of women with Hepatitis B Virus e Antigen (HBeAg) positive and negative☆

Agus Priyo Wibowo[a,*], Rina Masadah[a], Berti J. Nelwana[a], Djumadi Achmad[a], Gunawan Arsyadi[a], Maisuri T. Chalid[b], Andi Alfian Zainuddin[c]

[a] *Department of Pathology Anatomy, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia*
[b] *Department of Obstetrics and Gynecology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia*
[c] *Department of Public Health, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia*

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Abstract
Objective: This study aimed to determine the relationship between Asialoglycoprotein receptor (ASGP-R) on the placenta and positivity of HBeAg in the mother's serum.
Method: We collected 52 placentas from delivered mothers who have HbsAg-positive serum. The HbsAg-positive serum was then examined for HBeAg-positive and HBeAg-negative. Immunohistochemistry staining was performed on block paraffin sections using monoclonal antibody of ASGP-R.
Results: The expression of ASGP-R of 52 placenta samples demonstrated that 37 samples were scored I, five samples were score II, two samples were score III, and eight samples were score IV. We found that 14 of 52 serum samples were HBeAg-positive and 38 were HBeAg-negative.
Conclusion: There is a significant correlation between ASGP-R on the placenta and positivity of HBeAg in the mother's serum. The expression of the ASGP-R could increase the risk of HBV transmission. The result of this study could be used as a guideline for preventing and therapeutic approach of HBV from mother to child.

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* Corresponding author.
E-mail address: pmc@agri.unhas.ac.id (A.P. Wibowo).

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Introduction

In Indonesia, there are 5.3 million pregnant women every year, and about 2.7% pregnant women infected by Hepatitis B, so every year, there is an estimated 150 thousand babies suffer from hepatitis B which may develop into cirrhosis or hepatocellular carcinoma in the next 30 years.[1] There is a failure of prophylactic immunization even though it has been carried out adequately called a residual risk, where 3–5% of individuals will still get HBV after adequate prophylactic immunization procedures are carried out.[2] High viral load and HBeAg-positive are the main factors in the occurrence of residual risk.[2] HBeAg protein can cross the placental barrier and transplacental HBeAg from HBeAg-positive mothers makes neonatal T-helper cells unresponsive to the Hepatitis B Virus which is called immunotolerant.[3] This immunotolerant situation lasted for years in infants. It is one explanation for that 90% of mothers of HBeAg-positive carriers become chronic carriers.[3]

Asialoglycoprotein receptor (ASGP-R) is a membrane protein, that majority expressed on the surface of mammalian hepatocytes.[4] This receptor could interact with the preS1 domain of HBV.[5–8] ASGP-R binds glycosylated ligands and the preS1 domain of HBV is a glycoprotein. In vivo study on mice proves that binding ASGP-R with asialo-IFN-β presented larger suppression of HBV replication by inhibition of protein synthesis.[9]

Study of Vyas et al. showed an increase in the expression of ASGP-R on trophoblast cells and dendritic cells.[10] The other study showed that ASGP-R also expresses on peripheral blood monocyte and other inflammation cells.[11,12] This finding can be a role in vertical HBV transmission from mother to child. Inhibition of this receptor can be an effective strategy in eliminating transmission and preventing chronicity and complication on HBV infection.[10]

Method

This study was a cross-sectional analytic study to compare the expression of placental ASGP-R between HBeAg-positive group and HBeAg-negative group from HBsAg-positive mothers who had given birth. Maternal and placental blood samples were collected between the period of December 2017 and January 2018 from 1056 pregnant women who had given birth. With the criteria of mothers with HBsAg-positive serum, we obtained 52 placental and blood samples. Serum HBeAg was examined used ELFA method and the placenta sample processed into paraffin blocks for immunohistochemical staining of the Asialoglycoprotein receptor. This study has been approved by the Ethics Committee of the Faculty of Medicine, Hasanuddin University, Makassar, Indonesia (Number: 457/UN4.6.4.5.3.1/PP36/2019).

Examination of serum HBsAg and HBeAg

HBsAg examination of serum is carried out using the Monolisa kit (Bio-Rad, Marnes-la-Coquette, France) with a qualitative method based on the sandwich enzyme immunoassay method. This method uses monoclonal and polyclonal antibodies, which can bind with various subtypes of HBsAg. Monolisa kit uses an automatic device with a cut-off of <1.0 as negative and cut-off of >1.0 confirmed as positive.[13] Serum HBeAg examination was performed using Vidas kit (Biomerieux SA, Marcy-'Etoile, France) that uses ELFA (Enzyme-Linked Fluorescent Assay) method. The analysis is processed using an automatic Vidas instrument, with a negative cut-off if the index value is <0.1 and positive if the index value is ≥0.1.[14]

Placenta sample process and immunohistochemical staining

We obtained 52 placental samples from the mother who had given birth with HBsAg-positive. Placenta samples taken with a minimum size of 2 × 2 cm, which includes fetal side and maternal side, are then processed to become paraffin blocks. Paraffin blocks were carried out with immunohistochemical staining of Asialoglycoprotein receptor with deparaffinization and rehydration procedures with ethanol 100%, 90%, 80%, and 70%. The antigen rehydration was taken with citrate buffer at 103 °C for 10 min, then incubated with 3% H_2O_2 for 10 min at room temperature for blocking endogenous peroxidase activity, rinsed three times each of 3 min in Tris buffer saline (TBS) and then incubated in 10% bovine serum albumin (BSA) at room temperature in a humidified space for 30 min.

Then the primary antibodies, anti-ASGPR1 (A-5, Santa Cruz, TX, USA) 5 μg/mL were applied to parts that had been restricted to PAP pen and incubated for 2 h at room temperature. The pieces were then washed three times for 3 min each with TBS and incubated with conjugated HRP-secondary antibodies for 30 min at room temperature. Then, they were washed and incubated with diaminobenzidine (DAB) substrate, then counterstained with hematoxylin, the final step was dehydrated and installed with DPX.[15]

Interpretation of immunohistochemical Asialoglycoprotein receptor on the placenta

Placental samples prepared with immunohistochemistry of the Asialoglycoprotein receptor were analyzed and then be scored. Scoring is based on the percentage of placenta area stained by immunohistochemistry of Asialoglycoprotein receptor, as described in previous research by Vyas et al., score I (<5%), score II (5–30%), score III (31–60%), and score IV (>60%).[10] Score I and score II are maternal placentas infected by the Hepatitis B Virus but do not have the potential to transmit the HBV from mother to infant, score III is the intermediate state, and score IV is maternal placentas infected by the HBV and potentially transmit the HBV from mother to infant.[16]

Statistical method

HBeAg status is an independent variable and the immunohistochemistry of the ASGP-R is a dependent variable. Both variables are categorical. These variables will be analyzed using Mann–Whitney *U* test with an expected count <5 more than 20%. We took the value of 2 tailed, with a significance value of $p < 0.05$.

Table 1 Frequency of maternal HBeAg serum ($n = 52$).

HBeAg	Frequency	Percent (%)
Negative	38	73.1
Positive	14	26.9

Table 2 Immunohistochemistry of Asialoglycoprotein placenta receptors ($n = 52$).

Score of IHC ASGPR	Frequency	Percent (%)
I	37	71.2
II	5	9.6
III	2	3.8
IV	8	15.4

Results

The results of the study of 52 serum samples with positive HBsAg obtained data on serum HBeAg frequency in pregnant women, as shown in Table 1.

On examination of 52 placenta samples with immunohistochemistry Asialoglycoprotein receptors based on the criteria of HBsAg positive maternal serum, the following results were obtained, as shown in Table 2.

The 52 placenta samples that had been stained with Asialoglycoprotein receptor antibodies show that ASGP-R expressed on inflammatory cells and trophoblast cells. The results of the placenta staining with Asialoglycoprotein receptor immunohistochemistry as shown in Fig. 1.

The correlation between maternal serum HBeAg with immunohistochemistry of placental Asialoglycoprotein receptor as shown in Table 3. Mann–Whitney *U* statistical test was performed by comparing between HBeAg-positive and negative group with immunohistochemical expression scores of ASGP-R. The result was $p = 0.0001$ ($p < 0.05$), indicated that there was a significant relation between HBeAg serum with the expression of the ASGP-R on the placenta.

Discussion

The statistical test resulted in $p < 0.05$ state that there are significant relations between the HBeAg serum in the mother with the expression of ASGP-R on the placenta as shown in Table 3. This study reveals that there is an expression of ASGP-R on the placenta with varying degrees in pregnant women infected with HBV without distinguishing acute and chronic phases with HBeAg-positive and HBeAg-negative. The presence of ASGP-R expression score IV in the HBeAg-positive there is six samples and HBeAg-negative there two samples as shown in Fig. 1D, indicated that ASGP-R is expressed in replicating and non-replicating phase. These results show that there is potential transmission of HBV from mother to child. HBV replication can affect the expression of the Asialoglycoprotein receptor in the placenta of pregnant women by the up-regulation of cytokines in the JAK/STAT signaling pathway.[17,18] Pregnant women infected with HBV with HBeAg-positive or HBeAg-negative serum have the potential risk of transplacental transmission, but the potential for such transmission will be higher in HBeAg positive women.[3] Based on Vyas et al. study, the expression of ASGP-R on placenta by score I (Fig. 1A), score II (Fig. 1B), and score III (Fig. 1C), there is no potential risk for HBV transplacental transmission.[10] Transplacental transmission

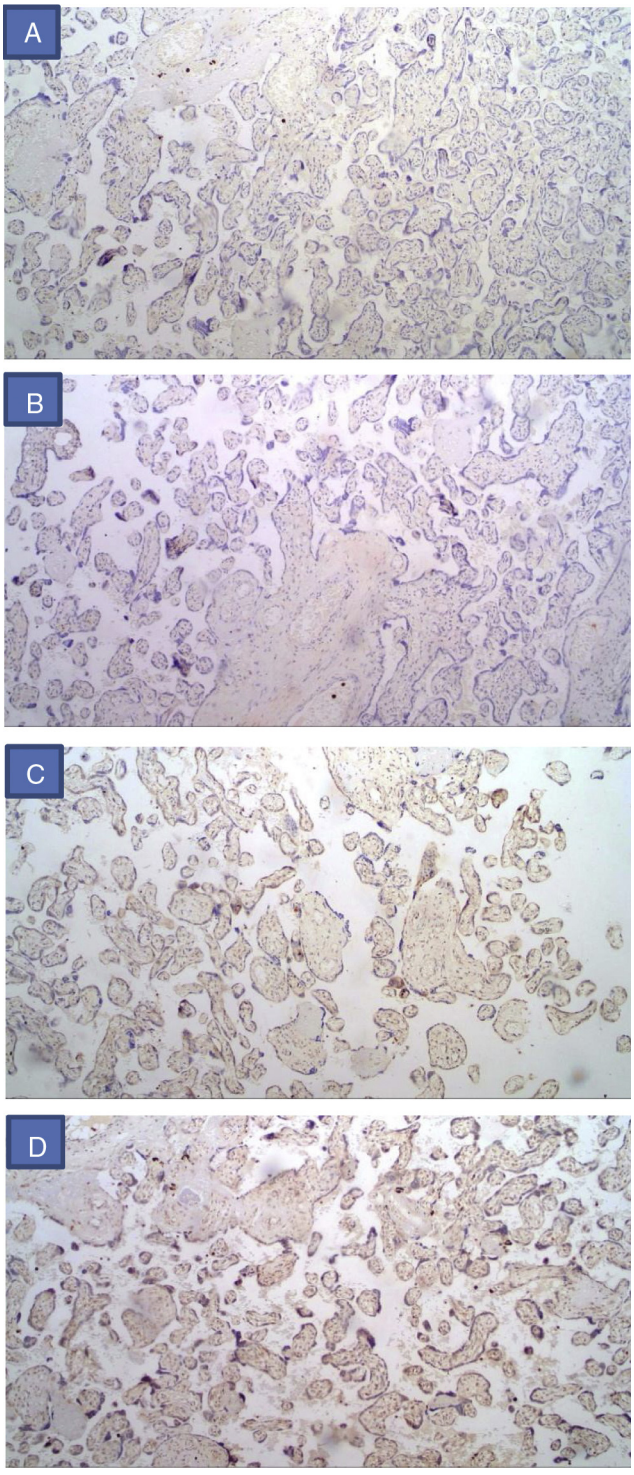

Figure 1 (A) Score I: ASGP-R expressed <5% in inflammatory cells; (B) Score II: ASGP-R expressed 5–30% in trophoblast cells and inflammatory cells; (C) Score III: ASGP-R expressed 30–60% in trophoblast cells and inflammatory cells; (D) Score IV: ASGP-R expressed 30–60% in trophoblast cells and inflammatory cells.

Table 3 The relationship between maternal serum HBeAg with immunohistochemistry of placental Asialoglycoprotein receptor.

HBeAg	Score ASGP-R				Total	*p*-value
	I	II	III	IV		
Negative	32	4	0	2	38	0.0001
Positive	5	1	2	6	14	

of HBV is a factor that causes a residual risk. Infants born to mothers with transplacental routes will continue to be infected with hepatitis B, even though adequate prophylactic immunization has been carried out on these infants.[2] Thus, it should be considered that pregnant women who have infected with HBV with HBsAg-positive and also HBeAg-negative need to get the same attention. HBeAg-positive serum indicates that the virus is in a replication state and HBeAg-negative suggests that the virus in the inactive phase and can also experience pre-core mutation, so HBeAg is not produced in serum.[19]

Conclusion

There is a significant correlation between ASGP-R expression on the placenta and HBeAg positivity in the mother's serum. The expression of the ASGP-R could increase the risk of HBV transmission. The presence of ASGP-R expression score IV on the placenta of pregnant women with HBsAg-positive, HBeAg-positive and HBeAg-negative can be used as a marker for vertical transmission HBV from mother to child. It needs more serious attention from a clinician for prophylactic and therapeutic interventions to infants.

Conflict of interest

The authors declare no conflict of interest.

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