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Correlation of FOXP3 Expression in Placenta as Regulatory T Cells Marker with Maternal HBeAg Serum and Intrauterine Transmission Status

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Abstract

Hepatitis B is an infectious disease caused by the Hepatitis B Virus (HBV) with a risk of transplacental (mother-to-child) transmission which is responsible for the failure of child's hepatitis B vaccination especially in mother with positive HBeAg serum. HBeAg plays a role in transplacental transmission by inducing in utero immunological tolerance by increasing the Regulatory T cells. This study aims to determine the expression of FOXP3 in the placenta as Regulatory T cells marker with positive and negative maternal HBeAg serum. This study was conducted in a cross-sectional study using 66 placental samples of mothers with positive HBsAg serum, group in positive and negative maternal HBeAg serum, also in positive HBV DNA serum with positive HBV DNA cord blood and positive HBV DNA serum with negative HBV DNA cord blood. FOXP3 expression was positive in 21 samples (31.8%), stained brown in Regulatory T cells. It was concluded that there was no significant difference between placental FOXP3 expression with maternal HBeAg serum and intrauterine transmission status (chi-square test, $p = 0.575$ and $p = 1{,}000$ ($p > 0.05$)).

Keywords: Hepatitis B; HBsAg; HBeAg; FOXP3; Regulatory T cell; Intrauterine Transmission; HBV DNA.

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1. Introduction

Hepatitis B Virus (HBV) infection is a world health problem that has a significant effect on socioeconomic condition [1,2]. More than 240 million of the world's population are infected with HBV and 40% among them are progressively developed into chronic hepatitis or/and hepatocellular carcinoma [3]. This risk is related to age at the time of infection. 90% of individuals who get perinatal HBV infection will develop chronic hepatitis. East Asia, Central Asia, Sub-Saharan, and the Pacific Region have a high prevalence of HBV infection during infant or young age (5-8%) [4-7]. In Asian countries, most HBV infection goes through vertical transmission from mother to child. The prevalence of chronic HBV infection in pregnant women with the risk of vertical transmission (Mother to Child Transmission) is 0.82% [6]. Mother to Child Transmission (MTCT) is responsible for more than one-third of chronic hepatitis B infections worldwide [7]. MTCT occurs more often in positive HBeAg serum and in high DNA viral serum level mothers [4]. Giving hepatitis B vaccine and hepatitis B immunoglobulin (HBIG) in newborns can prevent transmission during labor and postpartum. However, about 5-10% of the vertical transmission of HBV cannot be prevented because of the failure of protection against intrauterine infection. Unfortunately, there is no standard strategy during the gestational period to prevent this intrauterine transmission [8]. In some HBV endemic areas such as West Africa, transplacental transmission of the hepatitis B virus is very rare. However, in East Asia where genotypic B is endemic, the transplacental transmission is common, especially in seropositive HBeAg mothers [9]. Positive HBeAg serum can induce in utero immune tolerance because viral particles can cross the placental barrier. HBV infection obtained from the mother, in general, is in the immune tolerance stage until the patient becomes an adult. The exact mechanism of vertical transmission remains unclear, presumably, HBV transmission occurs perinatally at birth or in utero which results in immune tolerance status in infected infants [6,10]. Some studies suggested HBeAg causes chronic infection through in utero tolerance of T cells to HBV. HBeAg from positive HBsAg serum mothers inhibits T cells and dendritic cells function in fetal by increasing Regulatory T cells (Tregs) thereby reducing the immune response to HBV and increasing the risk of intrauterine transmission [7,11,12]. During HBV infection there is an increase in Tregs that cause suppression of T cells effector in immune tolerance patient. Infants born from mothers with chronic HBV infection have high circulating Tregs [6]. Forkhead Box Protein 3 (FOXP3) is widely used as a definitive marker of Tregs in patients with cancer and autoimmune diseases. FOXP3 is very important for the development and function of Tregs in mice and humans. In particular, the expression of FOXP3 in Tregs shows different function and phenotype between Tregs and T cells effector [13,14]. Tregs are produced through up-regulation of FOXP3 expression so there is a conversion from naive T cells to Tregs. FOXP3 inactivation causes Tregs deficiency [15]. The studies of the expression of FOXP3 in the placenta in correlation with HBV transmission have never been done before.

2. Materials and Methods

2.1.Collections of Samples

In this study, we obtained 66 placenta samples with positive maternal HBsAg serum from 1056 pregnant women during the period of December 2017 to August 2018 conducted in various hospitals in Makassar, Indonesia. Samples were then divided into two groups based on the data of maternal HBeAg serum by ELISA examination,

and the data of HBV DNA serum and HBV DNA cord blood examination by PCR. There were 11 samples with positive HBV DNA serum from 66 samples. These samples were undergone HBV DNA cord blood examination to see intrauterine transmission status; positive HBV DNA serum with positive HBV DNA cord blood indicates transmission, and positive HBV DNA serum with negative HBV DNA cord blood indicates no transmission.

2.2. Detection of FOXP3 in placental tissue by Immunohistochemistry

The placental tissue was processed into a paraffin block. The paraffin block was cut to size 5 µm, mounted to the poly-L-lysine object glass and then deparaffinized. Immunohistochemical staining used the standard method of Avidin-Biotin-Peroxidase Complex (ABC). Unstained slides were incubated with peroxidase-1 for 5 minutes at room temperature and followed with the ABC procedure. Immunohistochemical staining used concentrated FOXP3 monoclonal antibody with 1: 50 dilution. The positive control was done in parallel using tonsillar tissue. Positive cells were stained brown in Regulatory T cells, observed on a light microscope. Immunohistochemical staining results were evaluated by two pathologists.

2.3. Data Analysis

Data analysis was using a chi-square test to see the correlation between placental FOXP3 expression and maternal HBeAg serum status.

3. Result

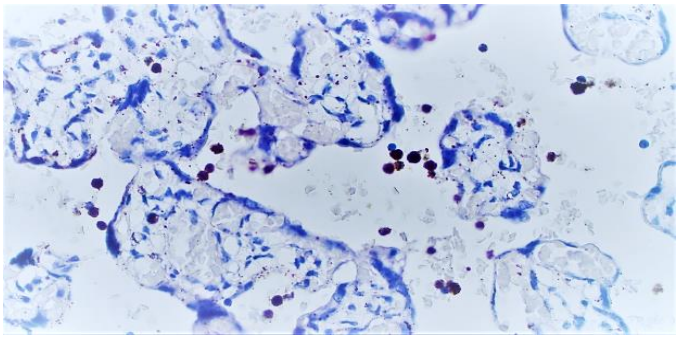

Figure 1: Positive Expression of FOXP3 only in Placental Lymphocytes (400X).

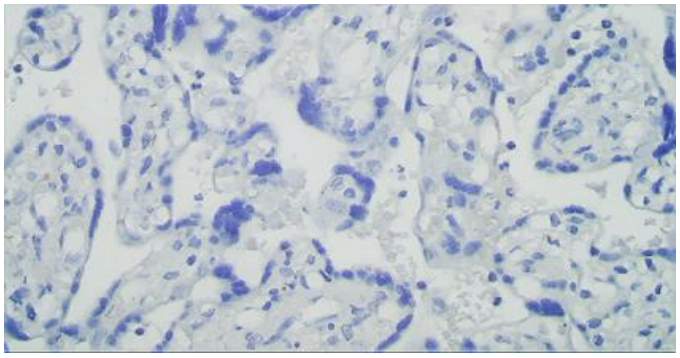

Figure 2: Negative expression of FOXP3 in both Trophoblast and Placental Lymphocytes (400X).

Table 1: Samples Distribution

	HBeAg Serum		FOXP3 Expression	
	N	%	N	%
Negative	50	75,8	45	68,2
Positive	16	24,2	21	31,8
Total	66	100	66	100

Based table 1, from placental samples of mothers with positive HBsAg serum, we obtained positive maternal HBeAg serum as many as 16 samples (24.2%) and negative HBeAg serum as many as 50 samples (75.8%), and positive FOXP3 expression as many as 21 samples (31.8%) and negative FOXP3 expression as many as 45 samples (68.2%).

Table 2: Correlation of Placental FOXP3 Expression with Maternal HBeAg Serum

	Negative FOXP3	Positive FOXP3	Total
Negative HBeAg	35 (70%)	15 (30%)	50 (100%)
Positive HBeAg	10 (62,5%)	6 (37,5%)	16 (100%)
Total	45 (68,2%)	21 (31,8%)	66 (100%)

Chi-square test

According to table 2, from 50 placenta samples of mother with negative HBeAg serum, there were 15 samples with positive FOXP3 expression (30%) and 35 samples with negative FOXP3 expression (70%), and from 16 placenta samples of mother with positive HBeAg serum, there were 6 samples with positive FOXP3 expression (37.5%) and 10 samples with negative FOXP3 expression (62.5%). Based on these, it was concluded that there was no correlation between the expression of FOXP3 in placenta with HBeAg serum status of mother (chi-square test, $p = 0.575$ ($p > 0.05$)).

Table 3: Correlation of Placental FOXP3 Expression with Intrauterine Transmission Status

	Positive HBV DNA Serum and Positive HBV DNA Cord Blood (Positive Intrauterine Transmission)	Positive HBV DNA Serum and Negative HBV DNA Cord Blood (Negative Intrauterine Transmission)	Total
Negative FOXP3	2(100%)	6 (66,7%)	8 (72,7%)
Positive FOXP3	0 (0%)	3 (33,3%)	3 (27,3%)
Total	2(100%)	9 (100%)	11 (100%)

Chi-square test

According to table 3, from 66 positive HBsAg mothers, 11 positive HBV DNA serum samples were obtained, consisted of 2 samples with positive HBV DNA cord blood and 9 samples with negative HBV DNA cord blood. Positive placental FOXP3 expression in positive HBV DNA serum and positive HBV DNA cord blood were 0 samples (0%) and negative placental FOXP3 expression with positive HBV DNA serum and positive HBV DNA cord blood were 2 samples (100%). Positive placental FOXP3 expression in positive HBV DNA serum and negative HBV DNA cord blood were 3 samples (66.7%) and negative placental FOXP3 expression with positive HBV DNA serum and negative HBV DNA cord blood were 6 samples (66.7%). Based on these data, it was concluded that there was also no correlation between expression of FOXP3 in placenta with intrauterine transmission status of mother (chi-square test, p = 1,000 (p> 0.05)).

4. Discussion

Hepatitis B is a global health problem and is the cause of chronic hepatitis, cirrhosis and liver cell carcinoma. According to WHO, hepatitis B is the second carcinogen in humans after tobacco cigarettes. The Hepatitis B virus can integrate with the placenta and cause infection. In the study of pregnant women with positive HBeAg serum, HBeAg can cross the placenta barrier. Positive HBsAg and HBeAg serum mothers tend to transmit to their children (70-90%) compared to positive HBsAg and negative HBeAg serum mothers [16]. In this study, we took placental samples from positive HBeAg-positive HBsAg serum and negative HBeAg-positive HBsAg serum mothers. Total positive HBsAg serum samples were 66 samples. From table 1, the number of positive HBeAg serum samples is 16 samples or 24.2% of the total sample. In a study of Wu and his colleagues in Taiwan, positive HBeAg serum from positive HBsAg serum mothers was 22% and this number decreased with the increasing of ages [17]. A study conducted by Tran and his colleagues showed that positive HBeAg serum was higher in women at reproductive age; 57.2% at age <44 years and 27.5%at age ≥ 45 years [18]. A study conducted in Ethiopia's Yirgalem Hospital obtained 38.2% positive HBeAg serum [19] dan in Ghana obtained 40% positive HBeAg serum of positive HBsAg serum mothers [20]. 84.2% perinatal infection occurs in infants born from positive HBeAg serum mothers and 8.7% in infants from negative HBeAg serum mothers. 90% of perinatal infections develop into chronic hepatitis [9]. Transmission from mother to child is the cause of perinatal infection. Hepatitis B virus transmission through the placenta is the least transmission way compared to infections that occur during labor and postpartum. The mechanism of HBV transmission in the uterus is still controversial. Some suspect transmission through the placenta occurs due to placental leakage, placental barrier damage, or through father and mononuclear blood cells in peripheral blood [16]. HBeAg is a replicative form of the Hepatitis B virus that can cross the placenta and induce in utero HBV-specific T cells tolerance. A study conducted by Shrivastava and his colleagues found that the number of regulatory T lymphocytes increased and dysfunctional CD8 T cells in infected infants and this played a role in the occurrence of persistent infections and suggested further research on the immunopathogenesis of HBV infections that were acquired in utero [10]. FOXP3 is a gene located on chromosome Xp11.23 which acts as a marker of Regulatory T cells and has immunomodulating functions [13,15,21]. FOXP3 is also related to cell development in humans. A number of previous studies have shown that the FOXP3 gene mutation is associated with autoimmune diseases. FOXP3 polymorphisms are associated with alopecia areata, habitual abortion, systemic lupus erythematosus, and

allergic rhinitis, also with susceptibility to preeclampsia [15]. FOXP3 expression can be triggered by various stimuli such as autoantigen to T cells, interactions with microbial metabolites, Transforming Growth Factor-beta (TGF-β) and Interleukin-10 (IL-10). Activation of FOXP3 protein inhibits the interaction of Nuclear Factor of Activated T cells (NFAT) and Nuclear Factor-kappa beta (NF-kβ) with genes associated with cytokines such as Interleukin-4 (IL-4) and Interferon-gamma (IFN-γ) [22]. From table 2, there was no significant difference between placental FOXP3 expression with HBeAg serum mothers ($p = 0.575$ ($p > 0.05$)). Positive expression of FOXP3 in the placenta with negative HBeAg serum was 15 samples and with positive HBeAg serum was 6 samples. Although there was no statistically significant difference, FOXP3 of Tregs were more expressed in the placenta with negative HBeAg serum. This is because the normal pregnancy process also affects the expression of Tregs in the placenta.

Regulatory T lymphocytes have a central role in fetomaternal tolerance and their frequency is increasing in the fetomaternal interface. During pregnancy, the immunity of pregnant women decreases to maintain fetal development. Estrogen and progesterone hormones are influential in regulating the immune response. Progesterone is immunosuppressive by inducing type 2 helper T cells (Th2) to maintain pregnancy. Increased of Th2 cells increase the CD4 + CD25 + Tregs [23]. Hu and his colleagues study found that FOXP3 expressed in trophoblast cells and decidua of the first-trimester placenta. At the end of the trimester placenta, the expression is lower because at the end of pregnancy immunologic changes occur which are characterized by a pro-inflammatory state for the delivery process. So the decrease of FOXP3 expression causes a shift in immunologic status from an immunotolerant state to a pro-inflammatory state at the end of pregnancy [21]. In this study, FOXP3 was positively expressed only in lymphocyte cells and not expressed in the trophoblast and decidua cells. Low immunity of pregnant women can cause replication of the hepatitis B virus and increase the chance of HBV transmission to the baby [21].

Increased regulatory T cells because the physiological process of pregnancy can create a gap that is prone to prenatal infection [24]. In table 3, we correlated FOXP3 expression with intrauterine hepatitis B virus transmission and it was found that FOXP3 did not affect the intrauterine transmission of the hepatitis B virus, this is probably because of too few samples or because transmission of the hepatitis B virus in our sample did not occur through the placenta. There was also no difference in expression of FOXP3 regulatory T lymphocytes with HBeAg serum as mentioned before. So that there is a possibility of transplacental HBV infection transmission occurs through other immunosuppressive mechanisms or HBeAg does not affect placental immunosuppressive conditions because HBeAg particles are so small that they can cross the placental barrier and directly into the fetal blood circulation. The limitation of this study is the number of samples that are too small, so it is necessary to conduct research with a larger sample size.

5. Conclusion

In this study, we concluded that there was no significant difference between FOXP3 expression in placental with both maternal HBeAg serum and intrauterine transmission status, so that maternal HBeAg serum status did not affect the expression of FOXP3 in placenta as Regulatory T cells marker, and also FOXP3 did not play a role in intrauterine transmission of hepatitis B.

References

[1] Navabakhsh B, Mehrabi N, Estakhri A, Mohamadnejad M, Poustchi H. Hepatitis B Virus Infection during Pregnancy: Transmission and Prevention. Middle East J Dig Dis [Internet]. 2011;3(2):92–102.

[2] Voiculescu M. How far we are towards eradication of HBV infection. J Gastrointest Liver Dis. 2015;24(4):473–9.

[3] Tang LSY, Covert E, Wilson E, Kottilil S. Chronic Hepatitis B Infection. Jama [Internet]. 2018;319(17):1802.

[4] Sarpel D, Kushner T, Carter D, Huisman T, Chiu S, Dieterich D. Mother-To-child transmission of hepatitis B and C virus: Review of the epidemiology and current treatment options. Future Virol. 2018;13(1):43–52.

[5] Trehanpati N, Hissar S, Shrivastav S, Sarin SK. Immunological mechanisms of hepatitis B virus persistence in newborns. Indian J Med Res [Internet]. 2013;138(5):700–10.

[6] Vyas AK, Jindal A, Hissar S, Ramakrishna G, Trehanpati N. Immune balance in Hepatitis B Infection: Present and Future Therapies. Scand J Immunol. 2017;86(1):4–14.

[7] Nelson NP, Jamieson DJ, Murphy T V. Prevention of perinatal hepatitis B virus transmission. J Pediatric Infect Dis Soc. 2014;3(SUPPL1):7–12.

[8] Liu J, Feng Y, Wang J, Li X, Lei C, Jin D, et al. An “immune barrier” is formed in the placenta by hepatitis B immunoglobulin to protect the fetus from hepatitis B virus infection from the mother. Hum Vaccines Immunother. 2015;11(8):2068–76.

[9] Li Z, Hou X, Cao G. Is mother-to-infant transmission the most important factor for persistent HBV infection? Emerg Microbes Infect. 2015;4(5).

[10] Shrivastava S, Trehanpati N, Patra S, Kottilil S, Pande C, Trivedi SS, et al. Increased regulatory T cells and impaired functions of circulating CD8 T lymphocytes is associated with viral persistence in Hepatitis B virus-positive newborns. J Viral Hepat. 2013;20(8):582–91.

[11] Hao HY et all. Relationship between HBeAg from HBsAg positive mothers and its influence on HBV intrauterine transmission. PubMed. 2017;38(10):1410–4.

[12] Tran TT. Immune tolerant hepatitis B: A clinical dilemma. Gastroenterol Hepatol. 2011;7(8):511–6.

[13] Pereira LMS, Gomes STM, Ishak R, Vallinoto ACR. Regulatory T cell and forkhead box protein 3 as modulators of immune homeostasis. Front Immunol. 2017;8(MAY):1–24.

[14] Li W, Han J, Wu H. Regulatory T-cells promote hepatitis B virus infection and hepatocellular carcinoma progression. Chronic Dis Transl Med [Internet]. 2016;2(2):67–80.

[15] Chen X, Gan T, Liao Z, Chen S, Xiao J. Foxp3 (-/ATT) Polymorphism Contributes to the Susceptibility of Preeclampsia. PLoS One. 2013;8(4).

[16] Abdi F. Hepatitis B and pregnancy: An update review article. World J Obstet Gynecol [Internet]. 2015;4(1):1.

[17] Wu C, Hsu T, Kung F, Changchien C, Tsai C, Lu S. Changes in the Prevalence of HBsAg and HBeAg : a Study of 8696 Parturients in a Well Vaccinated Area. Sci Rep [Internet]. 2017;(March):1–7.

[18] Tran TT, Gordon SC, Fung S, Dinh P, Yee L, Martins B, et al. Hepatitis B e Antigen Status and Hepatitis B DNA Levels in Women of Childbearing Age with Chronic Hepatitis B Infection Screening

for Clinical Trials. 2015;1–11.

[19] Amsalu A, Ferede G, Eshetie S, Tadewos A, Assegu D. Prevalence, Infectivity, and Associated Risk Factors of Hepatitis B Virus among Pregnant Women in Yirgalem Hospital, Ethiopia : Implication of Screening to Control Mother-to-Child Transmission. 2018;2018.

[20] Luuse A, Dassah S, Lokpo S, Ameke L, Noagbe M, Adatara P, et al. Sero-prevalence of Hepatitis B surface antigen amongst on commercial use on. 2016;7:10–2.

[21] Hu X, Wang Y, Mor G, Liao A. Forkhead box P3 is selectively expressed in human trophoblasts and decreased in recurrent pregnancy loss. Placenta [Internet]. 2019;81(13):1–8.

[22] Pereira LMS, Graça S, Simone RS, Conde S, Demachki S, Monteiro JC, et al. The − 3279C > A and − 924A > G polymorphisms in the FOXP3 Gene Are Associated With Viral Load and Liver Enzyme Levels in Patients With Chronic Viral Liver Diseases. 2018;9(September):1–16.

[23] Erkers T, Stikvoort A, Uhlin M. Lymphocytes in Placental Tissues: Immune Regulation and Translational Possibilities for Immunotherapy. Stem Cells Int. 2017;2017.

[24] Rowe JH, Ertelt JM, Xin L, Way SS. Regulatory T cells and the immune pathogenesis of prenatal infection. Reproduction. 2013;146(6).