Institut Pasteur
Microbes and Infection 15 (2013) 409–415
www.elsevier.com/locate/micinf

Original article

Role of maternal viremia and placental infection in hepatitis B virus intrauterine transmission

Yong Chen [a,b,*], Lijuan Wang [a], Yunfang Xu [a], Xingxiang Liu [a], Shouzhong Li [c], Qiang Qian [d], Bin Hu [e], Aijun Zhou [f], Tianyan Chen [b], Yingren Zhao [b,**]

[a] *Institute of Liver Disease Research, Huai'an Fourth People's Hospital, Huai'an, Jiangsu Province, China*
[b] *Department of Infectious Diseases, First Affiliated Hospital of Medical College, Xi'an Jiaotong University, Xi'an 710047, Shaanxi Province, China*
[c] *Department of Gynaecology and Obstetrics, The Huaiyin Hospital, Huai'an, Jiangsu Province, China*
[d] *Siyang Kangda Hospital, Suqian 223700, Jiangsu Province, China*
[e] *Suqian WeiMin Hospital, Suqian 223700, Jiangsu Province, China*
[f] *Lianshui People's Hospital, Huai'an 223400, Jiangsu Province, China*

Received 28 September 2012; accepted 26 February 2013
Available online 14 March 2013

Abstract

The mechanism of intrauterine hepatitis B virus infection has not been established. In this study, venous blood, cord blood, and placental tissues from 171 chronic hepatitis B virus infected pregnant women were tested for hepatitis B surface antigen, hepatitis B core antigen, and hepatitis B virus DNA. We found that residence, mode of delivery, age, and number of gestational weeks of pregnant women were not correlated with intrauterine hepatitis B virus infection, while neonates of mothers who were hepatitis B s antigen positive and hepatitis B e antigen positive ($P < 0.01$) or who had high hepatitis B virus DNA levels ($\geq 10^6$ copies/ml) were more likely to get an intrauterine infection ($P < 0.01$). The hepatitis B virus infection rate in placental cell layers gradiently decreased from the mother's side to the fetus's side of the placenta, but the odds ratio value of correlation between placental hepatitis B virus infection and intrauterine infection gradiently increased. The way of intrauterine hepatitis B virus infection may be through a layer–layer transmission pathway, although the possibility of placental leakage cannot be excluded.

Keywords: HBV; Intrauterine infection; Cord blood; Risk factors

1. Introduction

Hepatitis B virus (HBV) infection is a serious public health problem with 350 million chronic HBV carriers worldwide [1]. China is in the intermediate prevalence region of HBV [2], where 30–50% of chronic HBV infections are caused by mother–infant transmissions [3]. Moreover, 85–95% of persons infected by HBV during the perinatal period or before reaching 1 year of age will become chronic HBV carriers [4]. Hepatitis B vaccine and hepatitis B immunoglobulin could effectively prevent HBV intrapartum and postpartum transmission, but they have limited effects on HBV intrauterine infection [5,6]. Studies on intrauterine HBV infection show conflicting results. Some reports showed that intrauterine HBV infection was highly correlated with placental leakage regardless of maternal viremia such as hepatitis B s antigen (HBsAg) titer, hepatitis B e antigen (HBeAg) positivity, or high HBV DNA level [7–10]. However, other reports indicated that, when mothers were HBsAg+, HBeAg+, or had high HBV DNA levels, their infants were more susceptible to intrauterine HBV infection even if the placenta was intact [11–14]. To understand the exact mechanism of intrauterine HBV infection, we determined the risk factors for intrauterine HBV infection and studied the possible mechanism of infection.

* Corresponding author. Institute of Liver Disease Research, Huai'an Fourth People's Hospital, Huai'an, Jiangsu Province, China. Tel.: +86 517 8378 2885; fax: +86 517 8378 2838.
** Corresponding author. Tel.: +86 29 8532 3634; fax: +86 29 8532 3647.
E-mail addresses: xxchenguo@163.com (Y. Chen), zhaoyingren@sohu.com (Y. Zhao).

1286-4579/$ - see front matter © 2013 Institut Pasteur. Published by Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.micinf.2013.02.008

2. Material and methods

2.1. Subjects and blood collection

Excluding pregnant women with co-infections (e.g., HBV and hepatitis C virus, human immunodeficiency virus, or tuberculosis) or other known infections, and received with immunoglobulin or antiviral therapy [15,16], a random sample of 171 chronic HBV-infected pregnant women who gave birth in Huai'an Fourth People's Hospital, Huaiyin Hospital, Huai'an First People's Hospital, Huai'an Maternity and Child Care Centers, Siyang Kangda Hospital, Siyang WeiMin Hospital, and Lianshui People's Hospital of Jiangsu Province in eastern China between October 2009 and October 2011 and their neonates were recruited into our study with informed consent. Venous blood of pregnant women at delivery and cord blood were collected. After delivery, neonates from HBV-infected pregnant women received hepatitis B immunoglobulin (HBIG, 200 IU) within 24 h, while all infants followed the immunity schedule with hepatitis B vaccine (10 μg) at 0, 1, and 6 months.

The study was approved by the ethics committee of involved hospitals.

2.2. Placental tissues

Following a revised method reported previously [11], sterile placental tissues were collected after delivery, contained all placental cell layers, and measured about 1.5 × 2 × 3 cm. The samples were immediately fixed in neutral buffered 10% formalin, dehydrated, embedded in paraffin, and serially sectioned at 4 μm or 10 μm thickness. We included 157 placental tissues from 171 HBV-infected pregnant women in this study on placental HBV infection.

2.3. Testing of HBV markers in serum

HBV markers (HBVM) were detected by time-resolved fluoroimmunoassay (TRFIA). Simply, 100 μl samples were added to microplates, incubated for 40 min with slow oscillation, and washed 4 times. Then 100 μl of Europium-labeled working solution were added, incubated for 40 min with slow oscillation, and washed 6 times. Subsequently, 100 μl of fluorescence intensifier were added and incubated for 5 min with slow oscillation; finally, fluorescence was detected using Anytest 2000 (PekinElmer, Massachusetts, USA). Normal range of HBsAg is 0–0.2 ng/ml, HBsAb is 0–10 mIU/ml, HBeAg is 0–0.5 PEIU/ml, HBeAb is 0–0.2 PEIU/ml, and HBcAb is 0–0.9 PEIU/ml. If the level exceeded the maximum, the marker in the sera was considered positive.

2.4. Testing of HBV DNA in serum

HBV DNA was determined by commercially available real-time quantitative polymerase chain reaction (RT-qPCR) assay. All reagents and protocols were from Applied Biosystems, Inc. (USA). Briefly, HBV DNA was extracted from the specimens, and 2 μl of the extract were added to the polymerase chain reaction tubes. The ABI Prism 7300 procedure was followed, and the results were given directly by the instrument.

As stated in the HBV DNA real-time PCR detection kit, HBV DNA level >1000 copies/ml was defined as positive; conversely, a level <1000 copies/ml was defined as negative. Maternal HBV DNA level $\geq 1.0 \times 10^6$ copies/ml was considered high; $\leq 10^4$ copies/ml was considered low [17].

2.5. Detecting of HBsAg, HBcAg, and HBV DNA in placental tissues

Paraffin sections of placental tissues (4 μm) were deparaffinized in xylene and rehydrated in graded ethanol; then HBsAg and HBcAg were routinely detected by immunohistochemistry (IHC) assay. All reagents and protocols were from Zhong Shan Co. (Beijing, China). Liver biopsy specimens (HBsAg- and HBcAg-positive) were used as positive control, placental tissues of healthy pregnant women as negative control, and phosphate-buffered saline solution (PBS) instead of the primary antibody was used as blank control.

2.6. Detecting HBV DNA in placental tissues by RT-qPCR and in-situ hybridization

In RT-qPCR, placental tissues must be pretreated as follows: five 10 μm paraffin sections of placental tissues were deparaffinized in xylene, rehydrated in graded ethanol, and immersed in double-distilled water. Then an equal volume of proteinase K solution [10 mmol/L Tris–Cl (pH 8.0), 10 mmol/L EDTA, 150 mmol/L NaCl, 0.5% SDS, 200 μg/ml proteinase K] was added and incubated at 56 °C overnight. Finally, the solution was centrifuged at 13,000 rpm for 2 min, and the supernatants were treated as the serum above. HBV DNA level >0 copy/ml was defined as positive, conversely, HBV DNA level <0 copy/ml was defined as negative.

Using in-situ hybridization (ISH), we determined HBV DNA with the biotin-labeled DNA probe. The probe and related regents all were from TBD Science, Inc. (Tianjin, China). Liver biopsy specimens (HBV DNA $> 10^7$ copies/ml) were used as positive control, placental tissues of healthy pregnant women as negative control, and the unlabeled probe instead of the positive was also used as negative control.

2.7. Diagnostic principle

2.7.1. Intrauterine HBV infection

The diagnostic principle for intrauterine infection was positive for both HBsAg and HBV DNA in cord blood [18,19].

2.7.2. HBV-infected placental tissues

Any positive HBsAg, HBcAg, or HBV DNA in placental tissues was defined as placental HBV infection.