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ORIGINAL ARTICLE

# Correlations between Breast Milk HBsAg, Serum HBsAg, and Serum HBV DNA in Women with Chronic HBV Infection

Xiaotong Zhao [1], Chao Meng [1], Yuanyuan Xu [1], Wukui Cao [2]

[1] *Clinical Laboratory of Tianjin Second People's Hospital, Nankai District, Tianjin, China*
[2] *Department of Liver Disease, Tianjin Second People's Hospital, Nankai District, Tianjin, China*

## SUMMARY

***Background:*** **The aim is to investigate the correlations between breast milk HBsAg and hepatitis B serological markers and HBV DNA.**
***Methods:*** **Two hundred and twelve women with chronic HBV infection were recruited in our hospital from March 2019 to October 2019. Enzyme-linked immunosorbent assay (ELISA) and chemiluminescence microparticle immunoassay (CMIA) were used to measure HBsAg content. Serum HBsAg was determined by electrochemiluminescence. Real-time PCR was used to detect serum HBV DNA. The chi-squared test and nonparametric test were used for statistical analysis. Spearman's rank correlation test was used to measure the degree of association between variables.**
***Results:*** **In comparison to patients positive for HbsAg, anti-HBe antibody, and anti-HBc antibody, the patients positive for HBsAg, HBeAg, and anti-HBc antibody had significantly higher breast milk HBsAg content. Besides, patients with higher serum HBsAg levels also had higher breast milk HBsAg content than those with medium and low serum HBsAg levels. Patients with higher serum HBV DNA had higher breast milk HBsAg content than those with medium and low serum HBV DNA. The correlation analysis revealed a positive correlation between serum HBsAg and serum HBV DNA. In patients with serum HBV DNA ≥ 20 IU/mL, breast milk HBsAg content was positively correlated to serum HBV DNA. Breast milk HBsAg content was positively correlated with the serum HBsAg level. Additionally, the sensitivity of ELISA was significantly lower than CMIA in the detection of breast milk HBsAg.**
***Conclusions:*** **HBsAg content in breast milk is positively correlated with serum HBsAg level and HBV DNA load. CMIA is more sensitive than ELISA in the detection of breast milk HBsAg. Serum HBsAg level and HBV DNA load are useful for forecasting breast milk HBsAg content.**
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**Correspondence:**
Meng Chao
Clinical Laboratory of
Tianjin Second People's Hospital
No. 7, South Sudi Road
Nankai District, Tianjin
China
Phone: +86 22-27468190
Email: 13920616471@163.com

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## INTRODUCTION

How to stop the vertical transmission of HBV in serum HBsAg-positive pregnant women is a major challenge in HBV prevention because conventional HBV vaccination may not protect newborns from perinatal HBV infection. Meng J [1] and other groups showed that the positive rate of serum HBsAg in women of childbearing age was more than 6.2%. Breastfeeding is one of the

possible ways of the postpartum spread of HBV. HBV DNA is the "gold standard" of HBV replication. At present, most studies focus on HBV DNA detection in breast milk. However, current HBV DNA detection methods are slow so it is quite difficult to generate detection reports in time. In this study, we used ELISA and CMIA to detect HBsAg in breast milk and evaluated the correlation between serum HBsAg and HBV DNA.

## MATERIALS AND METHODS

### Patients

We randomly selected 212 HBV-infected women aged 18 - 39 who gave birth in our hospital from March 2019 to October 2019. The enzyme-linked immunosorbent assay (ELISA) indicated they were positive for serum HBsAg, anti-HBc antibody, HBeAg, or anti-HBe antibody. Without other reported or diagnosed diseases, all of them met the diagnostic criteria [2] published in the 2010 edition of the guidelines for the prevention and treatment of chronic hepatitis B.

### Specimen collection

Before delivery, 3 to 5 mL of venous blood was withdrawn from the patients, followed by centrifugation at 3,000 rpm for 5 minutes and collection of sera. Then, 8 mL of breast milk was collected within 24 hours after delivery after the nipples and periphery were sterilized and washed with saline. All procedures were conducted under aseptic conditions.

### Detection methods

HBsAg was measured by the ELISA kit purchased from Yingke Xinchuang (Xiamen) Technology Co., Ltd., following the manufacturer's manual. The samples were read on a PHOmo Microplate Reader (Autobio Diagnostics Co., Ltd). A sample was regarded as positive when the OD/cutoff $\geq 1$.

HBsAg was also quantified by the chemiluminescence microparticle immunoassay (CMIA) using the Abbott hepatitis B virus surface antigen quantitative determination kit on an Abbott automatic immunoanalyzer ARCHITECT i2000SR.

HBV DNA was quantified by real-time PCR using the COBAS® TaqMan® HBV Test for use with the High Pure System (HPS) on a COBAS® TaqMan® 48 Analyzer. The detection threshold is 20 IU/mL.

### Statistical analysis

The SPSS V21.0 statistical software was used for data analysis. Because the quantitative data were not normally distributed, they were presented as median with the 25th percentile (P25) and 75th percentile (P75). The quantitative data in different groups were compared using nonparametric tests for statistical analysis. Spearman's rank correlation test was used to measure the degree of association between variables. A p-value $< 0.05$ was regarded to be statistically significant.

## RESULTS

As indicated in Table 1, in comparison with patients positive for HbsAg, anti-HBe antibody, and anti-HBc antibody (Group B), patients positive for HBsAg, HBeAg, and anti-HBc antibody (Group A) had a significantly higher positive rate of breast milk HBsAg. CMIA results indicated that breast milk HBsAg content in Group A was significantly higher than that in Group B ($p < 0.05$).

We divided all patients into three groups according to serum HBsAg levels: Low group ($\leq 1 \times 10^3$ IU/mL), medium group ($1 \times 10^3$ IU/mL to $1 \times 10^4$ IU/mL), and high group ($> 1 \times 10^4$ IU/mL). As seen in Table 2, ELISA data indicated that the low group and medium groups had a significantly lower positive rate of breast milk HBsAg than the high group ($p < 0.05$). And there was no significant difference between the low and medium groups ($p > 0.05$). CMIA data demonstrated lower breast milk HBsAg content in the low group and medium group compared with that in the high group ($p < 0.05$), while no significant difference between the medium group and low group was observed ($p > 0.05$).

We then divided the patients into two groups according to HBV DNA levels: negative group (< 20 IU/mL) and positive group ($\geq$ 20 IU/mL). ELISA and CMIA data showed no difference in the positive rate of breast milk HBsAg between the two groups ($p > 0.05$). The positive group was further divided into three groups: low group (20 to $< 2 \times 10^3$ IU/mL), medium group ($2 \times 10^3$ to $2 \times 10^6$ IU/mL), and high group ($\geq 2 \times 10^6$ IU/mL). As outlined in Table 3, ELISA results showed that the positive rates of breast milk HBsAg in the negative group and the low group were significantly lower than that in the high group ($p < 0.05$), while there was no significant difference among the negative group, the low group, and the medium group ($p > 0.05$). CMIA data indicated that the negative group, low group, and medium group had lower breast milk HBsAg than the high group ($p < 0.05$), whereas no significant difference existed among the negative group, low group, and medium group ($p > 0.05$).

### Correlation analysis

The data of breast milk HBsAg, serum HBsAg, and serum HBV DNA were not normally distributed. Spearman's rank correlation tests showed that serum HBsAg was correlated with serum HBV DNA load. For patients with serum HBV DNA higher than 20 IU/mL, breast milk HBsAg content was correlated with serum HBV DNA load. Furthermore, breast milk HBsAg content was correlated with serum HBsAg levels.

### Comparison of ELISA and CMIA

In ELISA, a sample was regarded as positive when the OD/cutoff $\geq 1$. In CMIA, a sample was regarded as pos-

**Table 1. Breast milk HBsAg content in patients.**

| Group | Patients | ELISA | | CMIA |
|---|---|---|---|---|
| | | Positive cases | Positive rate (%) | Value |
| Group A | 78 | 62 | 79.49 * | 6.46 (1.86, 19.33) * |
| Group B | 134 | 71 | 52.99 * | 1.23 (0.14, 5.62) * |

* p < 0.05%.

**Table 2. Breast milk HBsAg content in groups of distinct serum HBsAg levels.**

| Serum HBsAg | Patient | ELISA | | CMIA |
|---|---|---|---|---|
| | | Positive cases | Positive rate (%) | Value |
| Low | 35 | 14 | 40.00 * | 0.70 (0.06, 7.32) * |
| Medium | 91 | 51 | 56.04 * | 1.64 (0.29, 4.37) * |
| High | 86 | 69 | 80.23 | 6.44 (1.19, 20.09) |

* p < 0.05%.

**Table 3. Breast milk HBsAg content in groups of distinct HBV DNA levels.**

| HBV DNA | Patient | ELISA | | CMIA |
|---|---|---|---|---|
| | | Positive cases | Positive rate (%) | Value |
| Negative | 45 | 24 | 53.33 * | 2.90 (0.27, 4.64) * |
| Low | 91 | 52 | 57.14 * | 1.73 (0.25, 8.54) * |
| Medium | 56 | 40 | 71.43 * | 3.80 (0.82, 16.52) * |
| High | 20 | 18 | 90.00 | 7.58 (2.84, 29.81) |

* p < 0.05%.

**Table 4. Sensitivity of ELISA method and CMIA.**

| Group | Patient | Positive cases | Positive rate (%) | $\chi^2$ | p |
|---|---|---|---|---|---|
| ELISA | 212 | 133 | 62.74 | 49.74 | 0.000 |
| CMIA | 212 | 194 | 91.51 | | |

itive when the value ≥ 0.05 IU/mL. The positive rate of breast milk HBsAg measured by ELISA was significantly lower than that measured by CMIA ($p < 0.05$, Table 4).

## DISCUSSION

The World Health Organization (WHO) states that breastfeeding is not a way of perinatal HBV transmission and thus recommends breastfeeding even if a mother carries HBV. However, the risk of HBV transmission to unvaccinated neonates through breastfeeding is a concern when HBV load tends to increase in breast milk [3,4]. Montoya-Ferrer et al. estimated that an infant's in-

testinal mucosa is exposed to more than 1 million HBV particles per day after a daily intake of 300 mL of breast milk in the first few weeks after birth [5]. Therefore, clinical detection of HBV in breast milk is still necessary on some occasions.

An HBV DNA test usually takes a long time and thus could not give a quick and timely readout. Inexpensive ELISA is currently the popular HBV DNA detection method in clinical laboratories. CMIA is more sensitive than ELISA in viral detection. Therefore, we tried to use CMIA to quantify HBsAg in breast milk. The objective of this study is to evaluate the correlations among breast milk HBsAg, serum HBsAg, and serum HBV DNA load to estimate the risk of breastfeeding-mediated HBV transmission.

HBeAg is an indicator of active HBV replication. During HBV infection, serum HBeAg can turn from positive to negative. A negative HBeAg result indicates very minimal or no HBV replication, suggesting that HBV activity is under control *in vivo* [7]. Elevated HBeAg, which occurs at the early or active stage of infection, signifies the high contagious ability of HBV infection [8]. Therefore, HBeAg is also an indicator of infection severity, inflammatory reactions, contagious ability, and response to antiviral treatments. HBeAg-mediated immunomodulation may influence the chronic and persistent presence of HBV after intrauterine or perinatal transmission [9]. Accordingly, we selected HBeAg as the reference indicator to divide patients into group A and group B. Consistent with a previous report [10], our data suggest a relatively high probability of breast milk-mediated HBV transmission in women with high serum HBeAg.

HBsAg is an envelope glycoprotein and used for hepatitis B diagnosis and prevention. The dominant epitopes of HBsAg are located in the hydrophilic region (MHR) of the protein and recognized by antibodies produced by reactive B cells [11]. The amino acid 99 - 169 in the MHR region are exposed on the surfaces of virions and are targeted by vaccine-induced antibodies and laboratory detection antibodies [12]. Petrova M et al. reported the existence of HBsAg and HBV DNA in the breast milk of pregnant women with active HBV replication [13]. Moreover, another study described the positive correlation between HBsAg in primary milk and maternal blood HBsAg [14]. Our results are consistent with these reports. Furthermore, we grouped patients on the basis of serum HBsAg levels and found that patients with low or medium serum HBsAg levels had significantly lower breast milk HBsAg amounts than those with high serum HBsAg levels. There was no significant difference between the former two groups. The results suggest that high serum HBsAg is associated with high breast milk HBsAg, whereas low/medium serum HBsAg does not influence breast milk HBsAg.

When the patients were grouped according to serum HBV DNA, HBV DNA-negative patients and HBV DNA-positive patients had comparable breast milk HBsAg levels. Further dividing of HBV DNA-positive patients revealed that the negative group, low group, and medium group had lower breast milk HBsAg than the high group, whereas no significant difference existed among the negative group, low group, and medium group. The data suggest that breast milk HBsAg is increased when HBV is actively replicated *in vivo*, and low HBV replication in the blood has no remarkable impact on breast milk HBsAg. A Montoya-Ferrer et al. argued that HBV transmission through breast milk might be of great significance especially when HBV is actively replicated in the mother [5]. Our study thus supports this viewpoint.

In addition, the present study also showed that although breast milk HBsAg quantity measured by CMIA was correlated with HBV DNA-positive group, ELISA showed no significant difference in breast milk HBsAg content among patients with medium, low, and high serum HBV DNA. This is perhaps because amino acid replacement in commercial ELISA kits renders HBsAg undetectable [15]. However, further studies are needed to test this hypothesis. Our study suggests that CMIA is more sensitive in detecting breast milk HBsAg than ELISA. Therefore, when using ELISA to detect breast milk HBsAg, CMIA results can be used as references in combination with serum HBsAg levels and serum HBV DNA load. In this manner, breast milk HBsAg content can be comprehensively evaluated for guiding safer breastfeeding.

**Declaration of Interest:**
None.

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