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CLINICAL ARTICLE

# Spontaneous preterm birth in women with chronic hepatitis B virus infection

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ABSTRACT

*Objective:* To determine whether chronic hepatitis B virus (HBV) infection, as evidenced by serum levels of HBsAg and HBV DNA, is a risk factor for spontaneous preterm birth (SPB). *Method:* The prevalence of HBV infection and the SPB rate were prospectively investigated among 1826 pregnant women, 30.89% Albanian and the remainder of other European origins. *Results:* Overall, 70 (3.8%) of the women were chronically infected with HBV. HBsAg status was strongly linked to SPB, which incurred to 5 (7.3%) of 64 women with circulating HBsAg compared with 28 (1.6%) of 1703 without current HBV infection (odds ratio, 5.2; $P = 0.007$). SPB, however, was linked neither to HBsAg levels, nor to HBV DNA levels, nor to the presence or absence of viremia. *Conclusion:* Pregnant women were found to be at higher risk for SPB if they had circulating HBsAg, and the risk did not seem to be influenced by the levels of HBsAg or HBV DNA.


## 1. Introduction

Preterm birth, or a birth occurring before the 37th week of gestation, is the leading cause of perinatal morbidity and mortality in high-resource countries. Its frequency is about 12% to 13% in the United States and 5% to 9% in Europe and other developed regions. It may occur spontaneously, after labor with membranes intact or with preterm premature rupture of the membranes, or after labor induction or cesarean delivery for maternal or fetal indications [1]. About 80% of preterm births are spontaneous and their causes can include maternal infection or inflammation, uteroplacental ischemia or hemorrhage, uterine overdistension, and immunologically mediated processes [2]. Several maternal risk factors [1], pregnancy history and characteristics [1,2], and genetic, environmental, and social factors have been associated with spontaneous preterm birth (SPB) [1,3–5], but its mechanism has not yet been established.

Whereas it is well known that bacterial infections activate the innate immune system, and that the risk of SPB is higher in pregnant women with bacterial infections [1,3,6–8], a link between SPB and infection with HIV, herpes simplex virus, adenovirus, parvovirus, hepatitis C virus, or hepatitis B virus (HBV) is still in question [3].

Infection with HBV is transmitted via body fluids, usually sexually or because of syringe contamination, or perinatally. The proteins of clinical importance for this virus are HBV surface antigen (HBsAg), HBV envelope antigen (HBeAg), and HBV core antigen (HBcAg). The presence of HBsAg in the serum is a marker of a current HBV infection whereas antibodies against HBsAg (anti-HBs) signify recovery (or a successful response to vaccination). The presence of HBeAg in the serum is a marker of active viral replication, and it is accompanied by the detectable presence of serum HBV DNA.

The effect of chronic maternal HBV infection on pregnancy outcome has been retrospectively evaluated in some studies from Southeast Asia [9–11]. These studies suggest that a chronic HBV infection in the pregnant woman may contribute to premature birth because of low-grade inflammation due to the release of proinflamatory cytokines, but this is still under investigation. The effect of chronic maternal HBV infection on pregnancy has been rarely investigated in Europe, where the HBeAg-negative serologic status dominates.

We explored whether SPB was associated with chronic HBV infection in a multinational population of pregnant women without known risk factors for preterm delivery. We also looked for possible relationships between SPB and serum levels of HBsAg and HBV DNA among the women with chronic HBV infection.

## 2. Patients and methods

This study was conducted from September 1, 2008 to August 31, 2009, with 1826 consecutive women delivered at the Department of Obstetrics and Gynecology of Athens Maternal and Prenatal Hospital "Elena Venizelou." As part of standard prenatal care at our institution, all women are screened in the first trimester of pregnancy for circulating HBsAg, HBeAg, anti-HBs, anti-HBe, and anti-HBc as well as for antibodies

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to HCV and HIV. In this study, the presence of HBsAg, anti-HBs and anti-Hbc was investigated once more at delivery. If the woman was found positive, she was tested for HBe-Ag, anti-HBe and HBV DNA, and if HBV DNA was detected, it was determined quantitatively. The diagnosis of chronic HBV infection was based on the presence of HBsAg in the first trimester of pregnancy and at the time of delivery.

An SPB was defined as a spontaneous labor prior to 37 weeks of gestation, with or without rupture of membranes. Women were excluded if they had a multiple pregnancy; a known history of preterm birth; a condition, such as diabetes, hypertension, pre-eclampsia, or placental hemorrhage, that had complicated the pregnancy; or concurrent infections.

A total of 2.0 mL of serum was obtained at delivery from the women chronically infected with HBV and four 0.5-mL aliquots from each sample were kept at −80 °C until used. The presence of HBsAg was determined using an Architect automated analyzer (Abbott Laboratories, Abbott Park, Illinois, USA). Viral presence and load were determined using the automated Cobas TaqMan HBV Test (Roche Diagnostics [Basel, Switzerland], which has a detection limit of 8 IU/mL. After the blood samples were manually prepared, the device extracted HBV DNA, amplified it by polymerase chain reaction using HBV-specific complementary primers, and quantified the amplicons using the HBV quantitation standard.

Continuous variables are presented as mean and standard deviation and categorical variables as number and percentage. The Shapiro-Wilk criterion was used to test for normality. All variables were found to be normally distributed and therefore classic statistical tests were used. The mean differences between 2 groups were assessed by the *t* test after controlling for equality of variances using the Levene statistic. One-way analysis of variance was used to assess differences between continuous variables among more than 2 groups. The Pearson $\chi^2$ test was used to test for associations between 2 categorical variables. If more than 10% of the expected frequencies in the contingency tables were less than 5, the Fisher exact test was used. After using the Bonferroni correction to adjust for an excessive type I error due to the number of comparisons made, the significance level was set at $0.05/7 = 0.007$. $P<0.007$ was therefore considered significant. A multiple logistic regression analysis was also performed to evaluate the effects of the independent variables on SPB. Data were analyzed using STATA statistical software, version 9.0 (Stata, College Station, Texas, USA).

The study was performed in accordance with the Declaration of Helsinki and approved by the local Hospital Review Board and Ethics Committee. Written informed consent was obtained from all participants.

## 3. Results

A total of 1826 pregnant women were screened at first trimester and at delivery. More than half of them, 1023 (56%), were from Greece; 564 (30.9%) were from Albania, and 239 (13.1%) were from Eastern European countries (Russia, Romania, and Bulgaria). Relevant characteristics are presented in Table 1.

Overall, 70 (3.83%) of the women tested positive for HBsAg at delivery, and most (50 [71.42%]) were Albanian. The prevalence of the different serologic markers of HBV, obtained at the time of delivery, according to country of origin is also presented in Table 1.

Only 2 (2.85%) of the 70 women who tested positive for HBsAg also tested positive for HBeAg, but the remaining 68 (97.15%) tested positive for anti-HBe. About half (274 of 564 [48.6%]) of the Albanian women tested positive for anti-HBc, followed by women from Eastern European countries (51 of 239 [21.3%]), and Greek women (61 of 1023 [6%]). Overall, anti-HBc and anti-HBs antibodies, which are serologic markers of a past HBV infection with spontaneous recovery, were detected in 252 (13.8%) of the women, and isolated anti-HBs positivity, which indicates protection from vaccination, was detected in 361 (19.76%) of the women.

**Table 1**
Characteristics of the study population according to place of origin.[a]

| Characteristic | Greece (n = 1023) | Albania (n = 564) | Eastern Europe (n = 239) |
|---|---|---|---|
| Age, y | 30.67 ± 5.38 [b] | 26.32 ± 4.86 [b] | 29.65 ± 5.36 [b] |
| Tested positive for | | | |
| HbsAg | 12 (1.2) [b] | 50 (8.9) [b] | 8 (3.3) [b] |
| Anti-HBc | 61 (6.0) [b] | 274 (48.6) [b] | 51 (21.3) [b] |
| Anti-HBs | 348 (34.0) | 216 (38.4) | 50 (20.9) |
| HBV DNA | 6 (0.6) | 25 (4.4) | 3 (1.3) |
| Weight of the newborn at birth, g | 3207.5 ± 429.8 [c] | 3310.4 ± 428.3 | 3289.1 ± 474.2 |

[a] Values are given as mean ± SD or number (percentage). Values were calculated at the time of delivery.
[b] $P<0.001$ for all comparisons.
[c] $P<0.001$ in comparison with the other 2 groups.

None of the women was coinfected with HCV, HDV or HIV, and no cases of cirrhosis were documented.

Twenty-six women, none of whom HBV infected, were excluded from the final analysis, 15 because of a twin pregnancy, 6 because of diabetes of pregnancy, 4 because of cholestasis of pregnancy, and 1 because of pre-eclampsia. The final analysis therefore included 1800 women. Spontaneous premature birth occurred for 33 (1.83%) of these women, 1.5% of Greek, 1.6% of Albanian, and 3.8% of Eastern European origin ($P = 0.05$). As 7 (14%) of the 70 HBsAg-positive women experienced an SPB, compared with 28 (1.62%) of the 1730 HBsAg-negative women, there was a correlation between SPB and HBsAg-positive status ($P = 0.007$) (Table 2).

To further evaluate the effect of HBsAg on SPB, we constructed a multivariate model with maternal HBsAg status, age, nationality, and weight of the newborn as independent variables and SBP as the dependent variable. On multiple logistic regression analysis, the presence of HBsAg was positively associated with SPB (odds ratio, 5.24; 95% confidence interval, 1.39–19.79; $P = 0.02$. The relative risk of preterm birth was 5.24 times higher for the HBsAg-positive than for the HBsAg-negative women.

Serum samples obtained at delivery from 44 of the 70 HBsAg-positive women were available for HBsAg and HBV-DNA quantification (of these, 43 were HBeAg negative). The mean HBsAg level in these samples was 2150.53 IU/mL, and the mean HBV DNA level was 6716.48 IU/mL. The HBV DNA levels were higher than 2000 IU/mL in 7 (15.9%) of the 44 samples; between 100 and 2000 IU/mL in 18 (40.9%) of the samples; between 8 and 100 IU/mL, i.e., very low, in 9 (20.45%) of the samples; and undetectable, i.e, less than 8 IU/mL, in 10 (22.75%) of the samples. It is important to note that HBV-DNA levels were extremely high, 228 000.000 IU/mL, in the sample from the only HBeAg-positive woman. The serum levels of HBsAg and HBV DNA for the 44 women with chronic HBV infection whose blood samples at delivery were available for evaluation are presented in Fig. 1.

In these 44 HBsAg-positive women, preterm birth was not related to the presence or absence of viremia ($P = 0.58$) or to viral load ($P = 0.76$). Preterm delivery was recorded in 1 (14.3%) of the 7 women with HBV DNA levels higher than 2000 IU/mL, in 2 (7.4%) of the 27 of women with HBV DNA levels less than 2000 IU/mL, and in 2 (20%) of the 10 women with undetectable levels ($P = 0.99$). On the other hand we found a trend for a relationship between SPB and

**Table 2**
Preterm birth rates in the study population according to the place of origin.[a]

| Greece (n = 1000) | Albania (n = 563) | Eastern Europe (n = 237) |
|---|---|---|
| 15 (1.5) | 9 (1.6) | 9 (3.8) |

[a] Values are given as number (percentage).

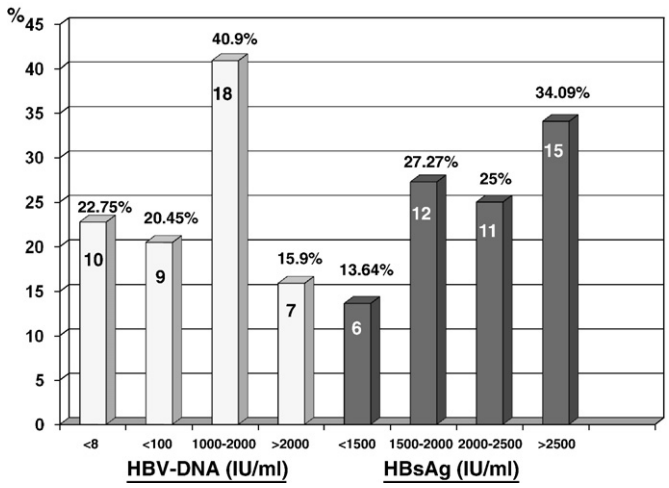


**Fig. 1.** Serum levels of HBV-DNA and HBsAg at delivery in the 44 women with chronic HBV infection.

HBsAg levels (2520.87 IU/mL for those who experienced an SPB vs 2120.51 IU/mL for those delivered at term), but the difference not reach statistical significance ($P = 0.08$).

## 4. Discussion

Compared with uninfected women, women with a chronic HBV infection were at higher risk of experiencing SPB, and those with higher serum HBsAg levels at delivery experienced SPB more frequently.

The prevalence of HBV infection differs around the world. It is low (<2% of the general population) in the United States, Canada, and Australia; intermediate (2%–8%) in Japan, Eastern Europe, and South America; and high (>8%) in the Far East, the larger part of Asia and Sub-Saharan Africa, and Western Europe. Historically, Greece has carried the highest burden of HBV infection in the European Union. The country implemented a hepatitis B prevention program in 1982, but it only targeted high-risk groups and had little impact on disease incidence or prevalence [12]. Over the past decade, however, HBV vaccination programs, demographic and socioeconomic changes, and the screening of blood donors have resulted in a significant decline in chronic HBV infection in Greece [13]. Nonetheless, the arrival of a great number of refugees, especially from countries where HBV infection is endemic, is likely to have slowed the decline; and the mother-to-infant (or vertical) transmission of HBV infection, which usually occurs in the perinatal period, is responsible for much of the disease burden in endemic areas [14].

In our study, 3.83% of the women were HBsAg-positive at delivery, and most (71.42%) were Albanian. Also, the vast majority of HBsAg-positive women (97.15%) were HBeAg-negative and anti-HBe–positive, which is consistent with reports recently published in Greece [15]. The dominance of the HBeAg-negative serologic status favours the efficacy of the immunoprophylactic program in our country, which is almost excellent among the offspring of women with low or undetectable HBV DNA levels during the perinatal period [16,17].

Although the perinatal transmission of HBV infection to the offspring of women with a chronic infection is a well-known phenomenon, data concerning the effect of chronic maternal infection on pregnancy outcome are limited and controversial. Most of the studies dealing with this topic were conducted in Southeast Asia, where the HBeAg-positive form of the infection and the HBV genotypes B and C dominate. In 1999, Wong et al. [11] concluded that the presence of HBsAg in pregnant women did not pose an additional risk to the pregnancy. Six years later, in a retrospective case-control study, Tse et al. [9] established that HBsAg carriers were at increased risk for threatened preterm labor, and suggested that the increased risk may be due to the chronic inflammatory state observed in these patients. In our study, SPB rate was low (1.83%), probably because the women with known risk factors for preterm birth were excluded from the analysis. Still, we found that the women testing positive for HBsAg were at significantly higher risk for SPB than those testing negative for this marker.

The HBsAg crosses the placental barrier in about one-third of all pregnant women with a chronic HBV infection who test negative for HBeAg, but this does not seem to cause passive or active immunoprophylaxis failure, or to play an important role in transplacental transmission of the virus [18]. On the other hand, the presence of HBsAg in the placental tissue and/or the cord blood, a phenomenon that is frequently observed in women with high serum HBsAg levels, may exaggerate the inflammatory response in these women and lead to obstetric complications. Many studies have shown that an increased serum concentration of proinflammatory cytokines plays an important role in premature labor and prelabor rupture of membranes. Our finding that SPB was more prevalent among women with chronic hepatitis B may be explained by their subclinical infection and a resulting systemic low-grade inflammation [6–8]. This low-grade inflammation response may also explain our observation that SPB was not related to serum levels of HBV DNA, a finding that needs further investigation. Besides, because HBsAg particles are produced much faster than complete virions, it is possible that the serum dilution occurring during pregnancy significantly affects HBV DNA levels but not HBsAg levels [19].

The current surveillance protocol being dedicated to HBV infection in our country, epidemiologic data are not collected. This is why this study presents no information concerning other infections of the genitourinary tract, and no environmental or social factors, such as smoking, alcohol consumption, nutrition, and economical status, that could affect the final results. Also, our study population came from of 3 different ethnic groups with different prevalence rates of hepatitis B, and was too small for us to perform subanalyses according to ethnicity. Focusing on groups with similar infection rates will be required to clarify the association between hepatitis B and SPB. The fact that 71.42% of the HBsAg carriers were Albanian women, even though they represented only 30.89% of our study population, may explain the rather wide 95% confidence interval for the odds ratio when we calculated the risks of SBP according to HBsAg levels. Finally, owing to the small number of women with detectable HBV DNA who incurred SPB, our results should be interpreted with caution and verified in larger prospective trials. Nevertheless, we believe that the large sample size of pregnant women with a relatively low risk of incurring SPB, and the fact that the study was conducted at a single center, where all tests were performed using the same methods, outweigh the drawbacks of the study. In conclusion, HBsAg-positive pregnant women appear to be at increased risk for incurring SPB, and this risk does not correlate with the serum levels of HBsAg or HBV DNA at delivery.

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## Conflict of interest

The authors declare that they nave no conflict of interest.

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