Maternal pre-pregnancy infection with hepatitis B virus and the risk of preterm birth: a population-based cohort study

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Summary

Background Preterm birth is the leading cause of child death in children younger than 5 years. Large cohort studies in developed countries have shown that maternal hepatitis B virus infection is associated with preterm birth, but there is little reliable evidence from China and other developing countries, where hepatitis B virus prevalence is intermediate or high. Hence, we designed this study to investigate the association between pre-pregnancy hepatitis B virus infection and risk of preterm and early preterm birth.

Methods Between Jan 1, 2010, and Dec 31, 2012, we did a population-based cohort study using data from 489 965 rural women aged 21–49 years who had singleton livebirths from 220 counties of China who participated in the National Free Preconception Health Examination Project. Participants were divided into three groups according to their pre-pregnancy status of hepatitis B virus infection: women uninfected with hepatitis B virus (control group), women who were HBsAg positive and HBeAg negative (exposure group 1), and women who were both HBsAg and HBeAg positive (exposure group 2). The primary outcome was preterm birth (gestation at less than 37 weeks). We used log-binomial regression to estimate adjusted risk ratios (aRR) of preterm birth for women with pre-pregnancy hepatitis B virus infection, and risk of early preterm birth (gestation less than 34 weeks).

Findings 489 965 women met inclusion criteria and were included in this study; of these, 20 827 (4·3%) were infected with hepatitis B virus. Compared with women who were not infected with hepatitis B virus, women who were HBsAg positive and HBeAg negative had a 26% higher risk of preterm birth (aRR 1·26, 95% CI 1·18–1·34) and women who were both HBsAg and HBeAg positive had a 20% higher risk of preterm birth (aRR 1·20, 1·08–1·32). Compared with women who were not infected with hepatitis B virus, women who were HBsAg positive and HBeAg negative manifested an 18% higher risk of early preterm birth (gestation less than 34 weeks; aRR 1·18, 1·04–1·34) and women who were both HBsAg and HBeAg positive had a 34% higher risk of early preterm birth (aRR 1·34, 1·10–1·61). Maternal pre-pregnancy hepatitis B virus infection was independently associated with higher risk of preterm birth and early preterm birth. These associations were similar in subgroups of participants as defined by baseline characteristics.

Interpretation Besides mother-to-child transmission, the risk of preterm birth in women infected with hepatitis B virus should not be neglected. Comprehensive programmes that focus on early detection of hepatitis B virus infection before pregnancy and provide appropriate medical intervention for women infected with hepatitis B virus before and during pregnancy would be helpful in improving maternal and neonatal outcomes and reducing child mortality.

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Introduction

Preterm birth, a birth occurring before 37 completed weeks of gestation, is the leading cause of death in children younger than 5 years.[1] An estimated 14·9 million babies, 11·1% of all livebirths worldwide, were born preterm in 2010.[2] Preterm birth complications are estimated to be responsible for roughly 35% of the world's 2·76 million annual neonatal deaths,[1] and surviving preterm babies are at increased risk of neurodevelopmental impairments and respiratory and gastrointestinal complications.[3] In low-income and middle-income countries, babies born before 34 weeks of gestation (early preterm birth) have only a 50% chance of survival[4,5] and the risk of maternal complications after early preterm birth is substantial.[6] Identifying risk factors is crucial for effective prevention, especially for early preterm birth. Several maternal factors have been associated with preterm birth, such as demographic characteristics, nutritional status, pregnancy history and current characteristics, infection, adverse behaviours, uterine contractions, and cervical length.[3] Among these factors, viral infection is an important cause of preterm birth. Previous studies have reported that maternal hepatitis B virus infection was associated with an increasing risk of preterm birth,[7–14] but some studies showed inconsistent results.[15–18] The association between hepatitis B virus infection and preterm birth is still controversial.

Research in context

Evidence before the study

We searched the published literature for studies on the association between hepatitis B virus infection and preterm birth through PubMed using the terms "hepatitis B virus" and "preterm birth", including all the reports published in both English and Chinese before Sept 20, 2016. We identified two large cohort studies on the association between hepatitis B virus infection and preterm birth, but these studies were done in developed countries where the prevalence of hepatitis B virus infection was relatively low. We also identified several studies done in developing countries where the prevalence of hepatitis B virus infection was relatively high or intermediate. However, the results of these studies were controversial.

Added value of this study

In this large population-based cohort study, we examined the association between pre-pregnancy hepatitis B virus infection and risk of preterm birth in 489 965 women. To our knowledge, our study is the first explorative study of maternal pre-pregnancy hepatitis B virus infection and preterm birth in a large-scale cohort in China. Results from this study showed that maternal pre-pregnancy hepatitis B virus infection was independently associated with increased risk of preterm birth and with early preterm birth (gestation less than 34 weeks). Compared with women uninfected with hepatitis B virus, women who were infected with hepatitis B virus had a 20–26% higher risk of preterm birth. Our findings add to the improved understanding of prevention and treatment of preterm birth, which is a risk factor for mortality in children younger than 5 years.

Implications of all the available evidence

This study highlights that, besides mother-to-child transmission, the risk of preterm birth in women infected with hepatitis B virus should not be neglected. Comprehensive programmes that focus on the early detection of hepatitis B virus infection before pregnancy, monitor the risk of preterm birth, and provide appropriate medical intervention for women infected with hepatitis B virus during pregnancy would be helpful in improving maternal and neonatal outcomes and reducing child mortality. Future research should explore the underlying mechanisms of hepatitis B virus infection's effect on preterm birth and effective strategies to reduce the risk of preterm birth among infected mothers, especially in developing countries.

Hepatitis B virus infection is a major health problem, causing high mortality and societal burden worldwide.[19] China has the world's largest burden of hepatitis B virus infection, with an estimated 74·6 million people chronically infected.[20,21] The prevalence of hepatitis B virus infection in women of reproductive age and pregnant women in China has been estimated at 3·87–9·98%.[18,22–27] Additionally, China has the second largest number of preterm births worldwide, with 1·17 million each year. However, large cohort studies depicting the association between maternal hepatitis B virus infection and preterm birth have almost always been done in developed countries with a low prevalence of hepatitis B virus infection; little reliable evidence exists from China or other developing countries where the prevalence of hepatitis B virus infection is intermediate or high. Furthermore, little has been done to investigate the association between pre-pregnancy hepatitis B virus infection and preterm birth. From a preventive point of view, identifying this association is important because it is relatively easy to intervene before pregnancy, and it might provide more effective results with respect to the gestational outcome for women with hepatitis B virus infection.

We did a large population-based cohort study in China to examine the association between maternal pre-pregnancy hepatitis B virus infection and risk of preterm and early pre-term birth.

Methods

Study design and participants

We did a large population-based retrospective cohort study in women of reproductive age (21–49 years) who participated in the National Free Preconception Health Examination Project (NFPHEP) from Jan 1, 2010, to Dec 31, 2012, successfully became pregnant, and then had a singleton livebirth in 220 counties in 31 provinces. NFPHEP was launched by the Chinese National Health and Family Planning Commission and the Ministry of Finance in 2010, with the aim to provide free health examinations before conception, counselling services for reproductive couples who planned to become pregnant within the next 6 months, and follow-up of pregnancy outcomes in the rural areas. Project-related design, organisation, and implementation have been described previously.[28–30] This study was approved by the Institutional Review Board of the Chinese Association of Maternal and Child Health Studies. All participants provided written informed consent before enrolment.

Procedures

Staff from local communities gathered data on the willingness of reproductive couples regarding pregnancy and enrolled those couples who had already made plans to become pregnant. Trained local health workers used a standardised questionnaire to collect baseline information from women who participated in the NFPHEP, including demographic characteristics (age, education level, occupation, ethnicity, and address of residence); history of chronic disease (hypertension, diabetes, chronic nephritis, thyroid disease, heart disease, anaemia, and cancer); history of pregnancy (gravidity and parity); and history of adverse pregnancy outcomes (spontaneous abortion, induced abortion, stillbirth, and preterm birth). Data were inputted into a web-based

electronic data collection system and sent to the national office. Trained qualified local health workers then did pre-pregnancy physical examinations. They measured bodyweight and height using calibrated instruments. Body-mass index (BMI) was calculated by dividing the weight in kg by the square of the height in m. Blood samples were taken and immediately sent to accredited local laboratories affiliated with authorised medical institutions.

Serum samples were separated and stored at −30°C before analysis. All samples were tested for the presence of hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg) using ELISA kits. Local laboratories made their own choices regarding reagent kits, all of which were approved by the China Food and Drug Administration. All reagent kits selected by local laboratories were tested by the National Center of Clinical Laboratories for Quality Inspection and Detection, with reagents produced by Abbott (Abbott Park, IL, USA) as the reference standard. Sensitivity, specificity, and κ value of the selected reagents from all of the counties involved were all higher than 95%. Serum alanine aminotransferase quantification (the most commonly used enzyme in the assessment of liver function), was also done using the kinetic method within 2 h of sample arrival at local laboratories. Elevated alanine aminotransferase was defined as greater than 40 IU/L. The National Center of Clinical Laboratories for Quality Inspection and Detection was responsible for the external quality assessment biannually, and for quality control.[31]

HBsAg positivity indicated that a participant was infected with hepatitis B virus. If a participant was tested as being both HBsAg and HBeAg positive, this indicated that virus was actively replicating and the level of infectiousness was high. We then divided participants into three groups according to their pre-pregnancy status regarding HBsAg/HBeAg: women who were HBsAg negative, indicating no infection with hepatitis B virus (control group); women who were HBsAg positive and HBeAg negative (exposure group 1); and women who were both HBsAg and HBeAg positive (exposure group 2).

After pre-pregnancy physical examination, all participants were followed up by trained local health workers by telephone each month. Health workers interviewed women face-to-face or by telephone within 3 months after conception, recording their last menstrual period, cigarette consumption by the women themselves and their husbands (they were all married) during pregnancy, and alcohol consumption by the women during pregnancy. Women were also interviewed face-to-face or by telephone 1 month after delivery to collect information on hospitals where delivery took place. Local health workers then collected detailed information from the medical records at these local hospitals with respect to pregnancy outcomes, including current pregnancy outcome (normal birth, preterm birth, miscarriage, induced abortion, or stillbirth), delivery date, gestational weeks, and newborn information (singleton or multiple births). The study was terminated when women had preterm birth or other pregnancy outcomes (such as normal birth, spontaneous abortion, induced abortion or stillbirth), or when the study reached the end of the observation period (Dec 31, 2012).

Outcomes

The primary outcome was preterm birth. We defined preterm births as births delivered at gestational ages less than 37 weeks and early preterm births as births delivered at gestational ages less than 34 weeks. The proportion of preterm and early preterm births was measured in respect to all singleton livebirths.

Statistical analysis

We included all women who fitted all inclusion criteria. We calculated medians and IQRs for age. We used proportions to describe baseline characteristics of the participants, such as region and educational levels, and the χ^2 test to compare the distributions of pre-pregnancy status of hepatitis B virus infection according to different baseline characteristics.

According to the pre-pregnancy status of hepatitis B virus infection, participants were divided into three groups: women who were HBsAg negative, indicating no infection with hepatitis B virus (control group); women who were HBsAg positive and HBeAg negative (exposure group 1); and women who were both HBsAg and HBeAg positive (exposure group 2). Log-binomial regression models were used to estimate the risk ratios (RRs) and 95% CIs of preterm births for women with pre-pregnancy hepatitis B virus infection.

Multivariable models were used and adjusted for potential risk factors for preterm birth. To examine the robustness of our findings, we did several sensitivity analyses that adjusted for different covariates in the multivariable models. In model A, we adjusted for sociodemographic characteristics of women, including age (21–24 years, 25–29 years, 30–34 years, 35–39 years, or 40–49 years); level of education (primary school or below, junior high school, senior high school, or college or higher); ethnic origin (Han or others); occupation (farmers, workers, or others); and region (eastern, central, or western China). In model B, we additionally adjusted for history of pregnancy and history of adverse pregnancy outcomes, including first gestation (yes or no), primipara (yes or no), history of preterm birth (yes or no), history of miscarriage (yes or no), history of stillbirth (yes or no), or history of induced abortion (yes or no). In model C, in addition to those factors included in model B, we also adjusted for BMI categories before pregnancy (<18·5 kg/m², 18·5–23·9 kg/m², 24·0–27·9 kg/m², or ≥28·0 kg/m²), elevated alanine aminotransferase before pregnancy (presence or absence), smoking status of husbands during pregnancy (yes or no), smoking status of women during pregnancy (yes or no), and alcohol drinking status of

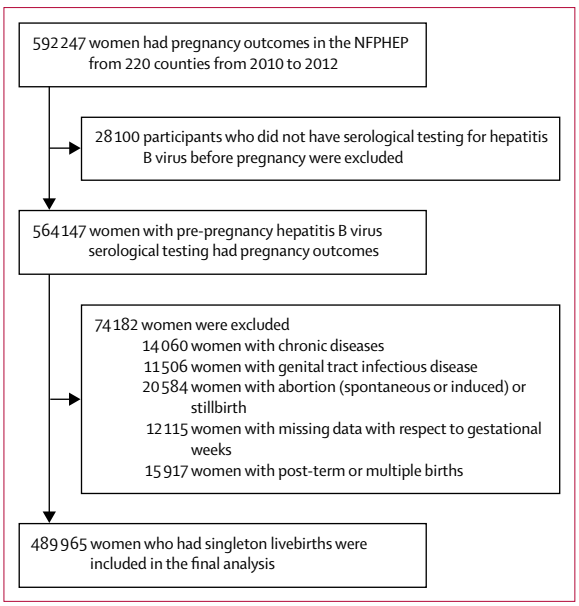

Figure 1: **Study profile**

See Online for appendix

women during pregnancy (yes or no). Adjusted RRs and 95% CIs of preterm births for women with pre-pregnancy hepatitis B virus infection were also calculated.

In the subgroup analysis, we divided women into different subgroups on the basis of baseline characteristics. Among these baseline subgroups, we examined the associations between maternal pre-pregnancy HBsAg status (regardless of HBeAg) and preterm birth after adjusting for other potential risk factors. All of the analyses were done with SAS software, version 9.4 and Stata software, version 14. Two-sided p values of less than 0·05 were deemed to be statistically significant.

Role of the funding source

The funder had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all data in the study and had final responsibility for the decision to submit for publication.

Results

By Dec 31, 2012, 592 247 women had pregnancy outcomes. We excluded 28 100 women who were not serologically tested for hepatitis B virus before pregnancy; 14 060 women with chronic diseases (including hypertension, diabetes, chronic nephritis, thyroid disease, heart disease, anaemia, and cancer); and 11 506 women with infectious disease of the genital tract. We excluded 20 584 women who had abortions, miscarriages, or stillbirths; 12 115 women with missing data on gestational weeks; and 15 917 women with multiple births or post-term pregnancies (gestation ≥42 weeks). The remaining 489 965 women were included in the final analysis (figure 1). The median age of all women included in the study was 26 years (IQR 24–29). 24·4% of the participants were older than 30 years, 69·4% had an education level of junior high school or lower, and 67·0% were in their first pregnancy.

Overall, 20 827 (4·3%) women had pre-pregnancy hepatitis B virus infection; 14 979 (3·1%) tested HBsAg positive and HBeAg negative, and 5848 (1·2%) tested both HBsAg and HBeAg positive. Compared with women who were uninfected with hepatitis B virus, those who were infected with HBV were more likely to be living in the eastern geographical region, underweight, and smoke or drink alcohol (table 1).

The median length of time from pre-pregnancy examination to pregnancy was 2·3 months (IQR 0·8–4·5). 25 766 of 489 965 women had preterm deliveries. The preterm birth rate was 5·26% (95% CI 5·20–5·32) among all the singleton livebirths. The preterm birth rate was 5·2% for women who were not infected with hepatitis B virus, 6·5% for women who were positive for HBsAg and negative for HBeAg, and 6·2% for women who were positive for both HBsAg and HBeAg. Multivariable adjusted analyses showed a significant association between maternal pre-pregnancy hepatitis B virus infection and preterm birth. The unadjusted and adjusted RRs (aRRs) are shown in the appendix. In the fully adjusted model, compared with women who were not infected with hepatitis B virus, women who were HBsAg positive and HBeAg negative had a 26% higher risk of preterm birth, and women who were both HBsAg and HBeAg positive had a 20% higher risk of preterm birth (table 2). Adjusting for different covariates did not substantially affect the estimates, which were similar for models A, B, and C (table 2).

Among the 489 965 women included in this study, 7019 (1·4%) showed early preterm delivery (gestation at less than 34 weeks). Early preterm birth occurred in 6656 (1·4%) women who were uninfected with hepatitis B virus, 251 (1·7%) women who were positive for HBsAg and negative for HBeAg, and 112 (1·9%) women who were positive for both HBsAg and HBeAg, and these trends were significant (χ_{trend}^2=15·866, df=1, p<0·0001). Multivariable adjusted analyses showed a significant association between maternal pre-pregnancy hepatitis B virus infection and early preterm birth. In the fully adjusted model, compared with women who were not infected with hepatitis B virus, women who were HBsAg positive and HBeAg negative had a 18% higher risk of early preterm birth and women who were both HBsAg and HBeAg positive had a 34% higher risk of early preterm birth (table 2). Adjusting for different covariates did not substantially influence the estimates, which were similar for all three models (table 2).

In the sensitivity analyses, the associations between hepatitis B virus infection and preterm birth did not change appreciably with additional adjustment for the length of time from pre-pregnancy examination to pregnancy (appendix p3); or exclusion of women self-reported

	Maternal pre-pregnancy status of hepatitis B virus infection*			Total (n=489 965)	Preterm birth (n=25 766)
	No infection (n=469 138)	HBsAg positive and HBeAg negative (n=14 979)	HBsAg positive and HBeAg positive (n=5848)		
Region					
Eastern China	114 114 (24·3%)	4010 (26·8%)	1527 (26·1%)	119 651 (24·4%)	5425
Central China	260 777 (55·6%)	7944 (53·0%)	3098 (53·0%)	271 819 (55·5%)	16 526
Western China	94 247 (20·1%)	3025 (20·2%)	1223 (20·9%)	98 495 (20·1%)	3815
Age (years)					
21–24	119 885 (25·6%)	3330 (22·2%)	1854 (31·7%)	125 069 (25·5%)	7012
25–29	234 966 (50·1%)	7531 (50·3%)	2988 (51·1%)	245 485 (50·1%)	12 562
30–34	85 622 (18·3%)	2942 (19·6%)	805 (13·8%)	89 369 (18·2%)	4650
35–39	22 350 (4·8%)	915 (6·1%)	158 (2·7%)	23 423 (4·8%)	1215
40–49	6315 (1·3%)	261 (1·7%)	43 (0·7%)	6619 (1·4%)	327
Education					
Primary school or below	20 676 (4·4%)	628 (4·2%)	260 (4·4%)	21 564 (4·4%)	1086
Junior high school	304 513 (64·9%)	9934 (66·3%)	3872 (66·2%)	318 319 (65·0%)	17 924
Senior high school	93 146 (19·9%)	2677 (17·9%)	1145 (19·6%)	96 968 (19·8%)	4176
College or higher	50 803 (10·8%)	1740 (11·6%)	571 (9·8%)	53 114 (10·8%)	2580
Occupation					
Farmer	355 920 (75·9%)	11 216 (74·9%)	4478 (76·6%)	371 614 (75·8%)	19 077
Worker	45 384 (9·7%)	1481 (9·9%)	611 (10·4%)	47 476 (9·7%)	3162
Other	67 834 (14·5%)	2282 (15·2%)	759 (13·0%)	70 875 (14·5%)	3527
Ethnic origin					
Han	443 739 (94·6%)	14 337 (95·7%)	5617 (96·0%)	463 693 (94·6%)	24 310
Other	25 399 (5·4%)	642 (4·3%)	231 (4·0%)	26 272 (5·4%)	1456
History of pregnancy					
History of preterm birth	783 (0·2%)	34 (0·2%)	12 (0·2%)	829 (0·2%)	68
History of miscarriage	11 257 (2·4%)	375 (2·5%)	121 (2·1%)	11 753 (2·4%)	730
History of stillbirth	3301 (0·7%)	121 (0·8%)	31 (0·5%)	3453 (0·7%)	228
History of induced abortion	58 785 (12·5%)	2134 (14·2%))	788 (13·5%)	61 707 (12·6%)	2849
First gestation	310 387/462 276 (67·1%)	9043/14 372 (62·9%)	3780/5558 (68·0%)	323 210/482 206 (67·0%)	16 382
Primipara	352 771/462 276 (76·3%)	10 486/14 372 (73·0%)	4408/5558 (79·3%)	367 665/482 206 (76·2%)	18 373
Pre-pregnancy physical examination					
Body-mass index (kg/m²)					
Underweight (<18·5)	60 922/458 316 (13·3%)	2093/14 293 (14·6%)	978/5535 (17·7%)	63 993/478 144 (13·4%)	3708
Normal weight (18·5–23·9)	345 486/458 316 (75·4%)	10 549/14 293 (73·8%)	3970/5535 (71·7%)	360 005/478 144 (75·3%)	18 589
Overweight (24·0–27·9)	43 440/458 316 (9·5%)	1385/14 293 (9·7%)	490/5535 (8·9%)	45 315/478 144 (9·5%)	2524
Obesity (≥28·0)	8468/458 316 (1·8%)	266/14 293 (1·9%)	97/5535 (1·8%)	8831/478 144 (1·8%)	486
Elevated alanine aminotransferase (>40 IU/L)	16 512 (3·5%)	1054 (7·0%)	948 (16·2%)	18 514 (3·8%)	1109
Lifestyle during early pregnancy					
Smokes	3518/465 003 (0·8%)	115/14 772 (0·8%)	48/5751 (0·8%)	3681/485 526 (0·8%)	245
Husband smokes	131 473/465 102 (28·3%)	3920/14 771 (26·5%)	1626/5748 (28·3%)	137 019/485 621 (28·2%)	7498
Drinks alcohol	4438/464 866 (1·0%)	150/14 772 (1·0%)	70/5749 (1·2%)	4658/485 387 (1·0%)	265

Denominators provided were some data were missing. *The distributions of pre-pregnancy status of hepatitis B virus infection with respect to different baseline characteristics were all statistically significant (p<0·05), except for smoking and alcohol drinking in women during pregnancy.

***Table 1*: Maternal baseline characteristics with respect to pre-pregnancy status of hepatitis B virus infection**

	Outcome	Model A*		Model B†		Model C‡	
		RR (95% CI)	p value	RR (95% CI)	p value	RR (95% CI)	p value
Preterm birth							
No infection	24 422 (5·2%)	1·00 (reference)	..	1·00 (reference)	..	1·00 (reference)	..
HBsAg positive and HBeAg negative	979 (6·5%)	1·26 (1·19–1·34)	<0·0001	1·26 (1·19–1·34)	<0·0001	1·26 (1·18–1·34)	<0·0001
HBsAg positive and HBeAg positive	365 (6·2%)	1·21 (1·09–1·33)	0·0002	1·22 (1·10–1·35)	0·0001	1·20 (1·08–1·32)	0·0006
Early preterm birth							
No infection	6656 (1·4%)	1·00 (reference)	..	1·00 (reference)	..	1·00 (reference)	..
HBsAg positive and HBeAg negative	251 (1·7%)	1·19 (1·05–1·35)	0·0059	1·18 (1·03–1·33)	0·0125	1·18 (1·04–1·34)	0·0095
HBsAg positive and HBeAg positive	112 (1·9%)	1·36 (1·12–1·63)	0·0011	1·38 (1·14–1·65)	0·0008	1·34 (1·10–1·61)	0·0025

RR=risk ratio. *Model A: risk ratios were adjusted for sociodemographic characteristics of women (age, level of education, ethnic origin, occupation, and region). †Model B: risk ratios were additionally adjusted for history of pregnancy (first gestation and primipara) and history of adverse pregnancy outcomes (preterm birth, spontaneous abortion, stillbirth, and induced abortion). ‡Model C: risk ratios were adjusted for pre-pregnancy body-mass index, pre-pregnancy elevated alanine aminotransferase, smoking status of husbands during pregnancy, and smoking and alcohol drinking status of women during pregnancy, in addition to the covariates in model B.

Table 2: **Adjusted RRs for preterm birth according to maternal pre-pregnancy status of hepatitis B virus infection**

with unreliable last menstrual period or inclusion of women with infectious disease of the genital tract; or exclusion of women followed up by telephones after delivery (data not shown).

Compared with women who were not infected with HBV, those who were HBsAg positive (regardless of HBeAg positivity) had a 24% higher risk of preterm birth (aRR 1·24, 95% CI 1·18–1·31; appendix). In the subgroup analyses, the associations between maternal pre-pregnancy hepatitis B virus infection and risk of preterm birth did not appear to be modified by baseline characteristics such as age, region, or educational level (figure 2). The association between maternal pre-pregnancy HBsAg positivity and risk of preterm birth appear to be somewhat greater with primipara or with first gestation.

Discussion

Preterm birth is one of the most common causes of mortality and morbidity in infants throughout the world, especially in low-income and middle-income countries.[32] In this large cohort of more than 490 000 women in China, we found that maternal infection with hepatitis B virus pre-pregnancy was associated with increased risk of preterm birth, and the associations were consistent for the various subgroups of participants defined by various baseline characteristics.

Investigators leading several large, retrospective cohort studies, which were mostly done in developed countries, assessed the association between maternal hepatitis B virus infection during pregnancy and the risk of preterm birth.[7,8] Reddick and colleagues[7] used data from the National Inpatient Sample from the Agency for Healthcare Research and Quality in the USA, encompassing 297 664 pregnancy-related discharges from 1995 to 2005, and found that women with hepatitis B virus had an increased risk of preterm birth compared with women with no hepatitis B virus infection (21·9% *vs* 12·1%; adjusted odds ratio [aOR] 1·65, 95% CI 1·3–2·0), after adjusting for maternal age, race, insurance status, substance use, sexually transmitted infections, and medical complications.[7] Sirilert and colleagues[12] analysed singleton pregnancies of 26 350 women in Thailand and found that the proportion of preterm births was significantly higher in pregnancies with positive HBsAg status (11·8% *vs* 10·0%; RR 1·013, 95% CI 1·001–1·0255), and preterm births were also significantly higher in women with positive HBeAg status (13·6% *vs* 8·6%; RR 1·250, 95% CI 1·000–1·563). Connell and colleagues[8] analysed all births in Florida, USA, from 1998 to 2007 using 1 670 369 birth certificate records linked to hospital discharge data, and found that women who were hepatitis B virus carriers were more likely to have infants born preterm than those who were not infected with hepatitis B virus in univariate analysis (10·78% *vs* 8·84%, p<0·0001), but they did not find such an association using multivariable analysis after adjusting for sociodemographic variables, parity, obstetric complications and delivery period (aOR 1·16, 95% CI 0·92–1·46).[8] The inconsistent results among previous studies might be related to the various characteristics within the study population, such as different prevalence of hepatitis B virus infection and preterm births. Some studies done in China did not find any association between maternal hepatitis B virus infection and preterm birth.[9,15,17,18] However, most of these studies were not large and some of them were case-control studies. The findings in our large cohort study were, however, consistent with most previous studies done in other countries, which found that maternal hepatitis B virus infection was associated with preterm birth.[7,10–12] The results of two meta-analyses showed that hepatitis B virus infection was not associated with placental abruption (OR 0·98, 95% CI 0·60–1·62),[33] and found a negative association between hepatitis B virus infection and pre-eclampsia (OR 0·77, 0·65–0·90).[34] The discrepancy of the association between pregnant complications and hepatitis B virus infection might be related to the

different characteristics of study population, methods of research, sample size, and regions.

The causes of preterm birth are complex and multifactorial. Although the precise mechanism of preterm birth cannot be established in most cases,[3] the association between maternal hepatitis B virus infection and preterm birth might be explained by the accumulation of hepatitis B virus DNA in the placenta and trophoblast cells that might initiate the placental inflammatory response, a known contributor to preterm birth.[12] Further study on the underlying mechanisms of hepatitis B virus infection-induced preterm birth is therefore required.

Our study also found that maternal pre-pregnancy hepatitis B virus infection was associated with an increased risk of early preterm birth (gestation less than 34 weeks), with a 18% higher risk in women who were HBsAg positive and HBeAg negative, and a 34% higher risk in women who were both HBsAg and HBeAg positive when compared with women uninfected with hepatitis B virus. The following studies are among the few pertaining to hepatitis B virus infection and early preterm birth. In 2005, Tse and colleagues[9] showed that the proportion of early preterm birth was higher for HBsAg-positive mothers than for HBsAg-negative mothers (4·7% *vs* 1·2%; p=0·033) in a case-control study of 506 Chinese women. In 2011, Elefsiniotis and colleagues[35] found that the presence of hepatitis B virus-DNA in cord blood was significantly associated with preterm birth in pregnant women with chronic hepatitis B virus infection, and the relative risk of hepatitis B virus DNA in cord blood was 6·43 times higher in women with serum hepatitis B virus DNA of at least 10 000 copies per mL and lymphocyte count of less than 1500 than in those with all the other combinations of both parameters (p=0·001). Individuals with HBeAg-positive chronic infection usually exhibit high levels of hepatitis B virus DNA and infectiousness, and our findings also indicated that early preterm birth was associated with higher levels of hepatitis B virus infectiousness in women. Whether a dose-response association exists between hepatitis B virus DNA load and early preterm birth needs to be further addressed in future studies.

Liveborn preterm babies drive the need for neonatal care, especially for early preterm birth. In low-income and many middle-income settings, preterm babies do not have even basic care, and such babies account for most preterm deaths worldwide.[2] There is only a 50% chance of survival for infants born before 34 weeks in low-income and middle-income countries.[2,36] From a public health perspective and with respect to policy and planning, prevention of preterm births is important, especially for early preterm births. Our study indicated that maternal

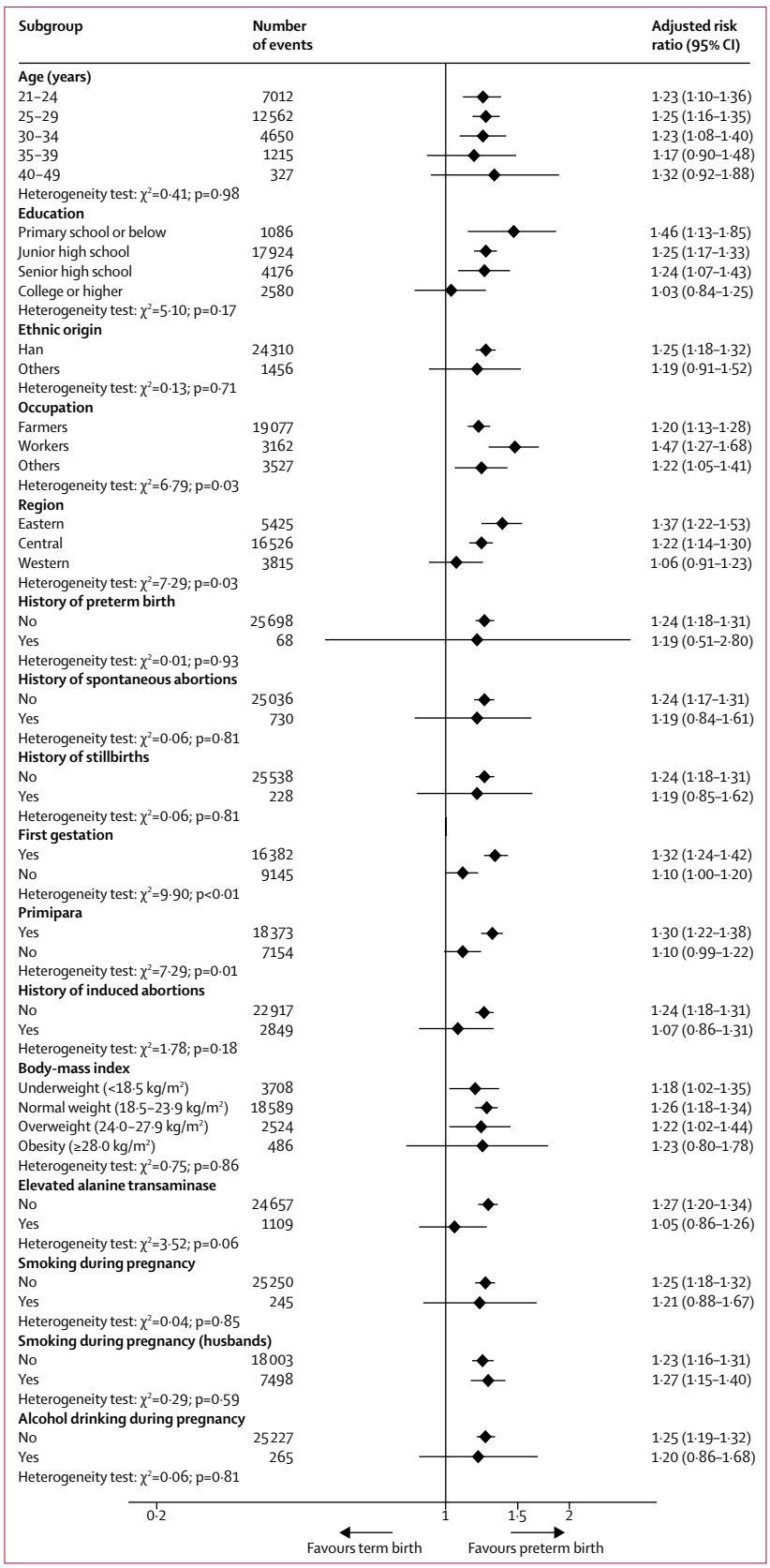

***Figure 2*: Subgroup analyses of associations between maternal pre-pregnancy HBsAG positivity (regardless of HBeAg) and risk of preterm birth**

pre-pregnancy hepatitis B virus infection was an independent risk factor of early preterm birth, and this risk was increased with the level of infectivity. These findings suggest that antiviral treatment might be considered (when necessary) for women who were infected with hepatitis B virus before pregnancy on the basis of their disease status and infectiousness to reduce the risk of preterm birth and to prevent mother-to-child transmission of hepatitis B virus; and monitoring the risk of preterm birth (especially for early preterm birth) by infected pregnant women should not be ignored.

This was the first explorative study of maternal pre-pregnancy hepatitis B virus infection and preterm birth in a large scale cohort in China. For this cohort, we recruited more than 490 000 participants, and followed up on pregnancy outcomes using strict quality controls. In this study, the number of events per variable was enough that the multivariable regression models were not over-fitted. The results showed that pre-pregnancy hepatitis B virus infection in women increased the risk of preterm birth and early preterm birth. However, our study has some limitations. First, hepatitis B virus load was not available for the NFPHEP project, and, therefore, women who were in the window period (ie, time between first infection and when the serological testing could reliably detect the infection) might not be detected and might be misclassified into the control group, which could lead to an underestimation of the associations between maternal hepatitis B virus infection and preterm birth. Meanwhile, dose-response associations between hepatitis B virus DNA load and preterm birth could not be ascertained. Second, the NFPHEP did not collect information on pregnancy complications such as gestational hypertension, gestational diabetes, premature rupture of membranes, or cervical incompetence. In the multivariable analysis, we could not adjust for these factors that might affect the associations between hepatitis B virus infection and preterm birth and we could not further assess the association between hepatitis B virus infection and different types of preterm birth.

Overall, in this large retrospective cohort study in rural China, maternal pre-pregnancy hepatitis B virus infection was associated with an increased risk of preterm birth. The NFPHEP potentially offers a unique platform to identify couples of a reproductive age living with hepatitis B virus infection who might not have progressed to late-stage liver disease,[37] and it also provides an opportunity to provide counselling for hepatitis B virus-infected women to prevent mother-to-child transmission and reduce the risk of preterm birth. Given the relatively limited health resources and heavy burden of hepatitis B virus infection in rural China, early detection of hepatitis B virus infection before pregnancy, monitoring the risk for preterm birth, and providing appropriate medical intervention for women infected with hepatitis B virus during pregnancy would be helpful in improving maternal and neonatal outcomes and reducing child mortality.

Contributors
JL and SZ searched the literature, designed the study, analysed the data, interpreted the results, and drafted the manuscript. QW, HS, and YZ collected the data and revised the manuscript. ML conceived, designed, and supervised the study, interpreted the results, and revised the manuscript. All authors contributed to the writing of the manuscript.

Declaration of interests
We declare no competing interests.

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