Maternal infection with hepatitis B virus before pregnancy and risk of congenital malformations in offspring: a record-linkage study of a large national sample from China

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Summary

Background Whether hepatitis B virus (HBV) infection of women prior to pregnancy can influence risk of congenital malformations in offspring remains controversial. We assessed the association between them by considering congenital malformations in the aggregate as well as risk of organs systems using a large national sample of Chinese women.

Methods We performed a record-linkage cohort study of women who participated in National Free Preconception Health Examination Project, between January 1, 2010, and December 31, 2019 for whom data on congenital malformations in their offspring were available from the National Population-Based Birth Defects Surveillance Network. A total of 498,968 linked records were obtained, of which 127,371 were excluded because HBV status before pregnancy was unknown, the records involved multiple pregnancies, or pre-pregnancy examinations were conducted after conception. Based on pre-pregnancy status, mothers were assigned to two categories of HBsAg– or HBsAg+ and, in certain analyses, to three categories of HBsAg–, HBsAg+/HBeAg– or HBsAg+/HBeAg+. Potential associations of serological status with risk of congenital malformations, considered separately or in aggregate, were explored using multilevel logistic regression. Factors that might influence such associations were also explored.

Findings Among the 371,597 women analyzed, 21,482 (5.78%) were HBsAg+ before pregnancy, and 8333 (2.24%) had a fetus or child diagnosed with congenital malformations, composed of 7744 HBsAg– women and 589 HBsAg+ women. HBsAg+ status was associated with increased risk of congenital malformations in the aggregate (OR 1.14, 95% CI 1.03–1.25) and of cardiovascular malformations specifically (OR 1.18, 95% CI 1.03–1.35). HBsAg+/HBeAg– status was associated with significantly higher risk of cardiovascular malformations (OR 1.19, 95% CI 1.01–1.39) as well as reproductive malformations (OR 1.51, 95% CI 1.02–2.23). Associations between HBsAg+ status before pregnancy and risk of congenital malformations was modified by alanine aminotransferase activity ($P_{interaction} < 0.05$).

Interpretation Prepregnancy HBV infection might be associated with fetal malformations. This association needs further investigation to confirm whether it is a causal association, and assess whether antiviral therapy of women with HBsAg+ planning to conceive might reduce the risk of fetal malformations.

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Research in context

Evidence before this study

We searched PubMed for relevant studies published before April 2024 using the search string *("hepatitis B virus" OR "HBV" OR "HBsAg" OR "HBeAg") AND ("birth defect" OR "congenital malformation" OR "congenital anomaly" OR "congenital abnormality" OR "congenital disorder") AND ("maternal" OR "pregnancy" OR "fetal" OR "fetus" OR "offspring")*. We also examined reference lists from suitable studies. We examined more than 150 publications, including 16 observational studies of the teratogenic effects of maternal HBV infection on offspring. Those studies came to divergent conclusions. A meta-analysis involving 16,205 pregnant women exposed to HBV in 14 studies, all published before 2021, suggested that HBV carrier status of the mother may increase risk of congenital malformations, but the available evidence still fails to draw a firm conclusion. While evidence supports a link between maternal exposure to HBV and higher risk of congenital malformations affecting the cardiac system, corresponding evidence is lacking about the risk of congenital malformations affecting other organ systems.

Added value of this study

Our study is the first-ever analysis of the association of maternal HBV carrier status before pregnancy with risk of congenital malformations in the aggregate and malformations affecting organ systems. The results of this study provide new evidence for further confirming the association between HBV carrier status and congenital malformations, and further suggest that HBsAg+/HBeAg− status was associated with higher risk of malformations affecting cardiovascular and reproductive systems.

Implications of all the available evidence

Our results are relevant to prevention of congenital malformations in offspring of women who are HBV carriers and provide much needed information about whether there exists an association between HBV carrier status and congenital malformations. Future studies should further confirm the causal relationship between them, and evaluate the dose–effect relationship between maternal HBV-DNA before pregnancy and occurrence of congenital malformations in offspring.

Introduction

Hepatitis B virus (HBV) is a small, enveloped, hepatotropic virus with a partly double-stranded, relaxed circular (rc) DNA genome; it is transmitted through exposure to infectious blood and other body fluids; and it transiently or chronically infects the liver.[1,2] Approximately 3.9% of the global population or around 300 million people are chronically infected with HBV,[3,4] and 15–40% of such individuals will develop cirrhosis, liver failure, or hepatocellular carcinoma.[5] In areas where the virus is endemic, the most frequent mode of transmission is *in utero*.[6] Approximately 4.8% of pregnant women worldwide and nearly 7% in areas such as Africa where the virus is endemic are seropositive for the hepatitis B surface antigen (HBsAg),[7,8] which usually indicates chronic infection. China is one of the countries with the largest HBV burden in the world, with approximately 87 million people affected.[9] In 2020, the HBV prevalence among pregnant women in mainland China is 5.44%.[10] In general, the HBV prevalence has declined in recent decades through public health efforts to halt transmission from mothers to babies during pregnancy and official recommendations to vaccinate babies against the virus.[11,12]

The prevalence of congenital malformations worldwide is around 2.12%–6.0%.[13,14] Approximately 50% of congenital malformations have unknown causes, but known factors include single gene defects, chromosomal disorders, multifactorial inheritance, environmental teratogens, and micronutrient deficiencies.[14] Extensive epidemiological evidence has linked HBV infection during pregnancy with higher risk of miscarriage,[15] preterm birth,[16] low birth weight[17] as well as fetal distress.[17] Whether it also increases risk of congenital malformations analogously to rubella virus, cytomegalovirus or herpesvirus[18] is unclear. Studies have established the possibility of multiple routes of prenatal HBV infection, including through placental infection, transplacental transmission from mother to fetus[19] and transmission of viral DNA from an infected oocyte or sperm into the fetus.[20–22] Several studies have focused on the association between maternal HBV infection and risk of fetal malformations, but their findings remain controversial,[15,23–25] which may reflect their relatively small samples of HBV infected women whose fetus were diagnosed with congenital malformations. Moreover, all previous studies focused on congenital heart

defects or unspecified congenital malformations and none has systematically evaluated the effect of maternal HBV infection on organ specific malformations.

We hypothesized that HBV infection before pregnancy would be associated with an increased risk of congenital malformations. Here we explored a potential link using a large, nationwide sample of Chinese women who were enrolled in the National Free Preconception Health Examination Project (NFPHEP), which prospectively collected data on serological status of women planning to become pregnant, and we linked these women to the outcome of their subsequent pregnancy in the National Population-Based Birth Defects Surveillance Network (NPBDSN). We estimated risk of congenital malformations in the aggregate as well as risk of organ specific malformations among HBV-infected mothers. The findings of this study may provide meaningful evidence for formulating more effective policies to reduce congenital malformations by improving the management of HBV-infected women planning to conceive.

Methods

Data sources and study population

In this record-linkage cohort study, we recruited women who were enrolled in the NFPHEP between January 1, 2010 and December 31, 2019 and delivered before December 31, 2020. The Chinese government launched NFPHEP to provide free health examinations to couples planning to become pregnant.[26] From 2010 until 2012, the Project covered 220 counties/districts in mainland China; from 2013, the Project covered 2907 counties/districts in all 31 provinces of mainland China.[27] Health workers from maternal and child health organizations operating at the county or district level used a structured questionnaire to collect information on the married couple's demographic characteristics, history of chronic disease and history of pregnancy. The workers also performed a routine physical examination of the woman, whose blood was sampled and promptly assayed at accredited clinical laboratories as described below. All these data on baseline information and results from physical and laboratory examinations were input into a web-based electronic data collection system and sent to the national office.

Pregnancy outcomes were obtained from the NPBDSN, which covers 64 counties/districts in 30 provinces, municipalities, or municipal districts in China (Figure S1).[28] Surveillance staff operating in the network are required to enroll all neonates born alive or dead after at least 28 gestational weeks to women who have lived in the surveillance area for at least one year, and to report any congenital malformations diagnosed by 42 days after birth. In order to facilitate data collection, a comprehensive surveillance network was established at the levels of community/township/village, county, city, province, and the country. Surveillance staff collected, verified and followed up on birth information at the level of community/township/village. Standardized forms were used to gather data on births and congenital malformations from hospital medical records, which were then reported to the county, city, and province. Data included the identity of mother and father, maternal ethnicity, maternal residence address, maternal age at delivery, parity, gestational age, number of fetuses, date of delivery, birth weight and sex, and outcomes at birth or within 42 days after birth (stillbirth, death or congenital malformations). Each level was accountable for verifying the data within its jurisdiction; doubtful data were flagged and returned for verification. At the national level, data were checked and encoded by clinicians, statisticians, epidemiologists, and information technicians at the National Maternal and Child Health Monitoring Office. This team classified all congenital malformations in the database according to the International Classification of Diseases (10th revision). The accuracy and completeness of the data were verified through cross-referencing with relevant data from various systems such as birth certificate registries and records of perinatal deaths. Furthermore, regular annual surveys were carried out to detect and rectify any errors or inaccuracies present in the gathered data. The rate of underreported live births or birth defects, as well as the rate of errors or missing values in the reporting forms, should not exceed 1%.

The present study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Sichuan University (K2017045, China). All participants provided written informed consent before enrolled in the NFPHEP. The study followed the reporting statement of STROBE guideline.[29]

Linkage of maternal records to records of birth outcomes

We extracted 41,697,696 records from the NFPHEP database covering the period from January 1, 2010 until December 31, 2019, and 741,672 records from the NPBDSN database covering the period from January 1, 2010 until December 31, 2020. After cleaning the data, records from the two databases involving the same woman were linked based on the national identity number, leading to 540,755 linked records pairs (births). For some women in the NFPHEP, data were available for multiple pre-pregnancy examinations, so only the data for the examinations closest to the estimated date of conception were included. In the end, 498,968 linked record pairs involving that number of mothers and births were analyzed in this study (Figure S2).

Blood analysis

Promptly after collection, blood was assayed at accredited clinical laboratories for the following: alanine aminotransferase (ALT) activity, HBsAg, HBeAg,

hepatitis B surface antibody (HBsAb), hepatitis B envelope antibody (HBeAb) and hepatitis B core antibody (HBcAb). Laboratory tests were conducted as described[16] using enzyme-linked immunosorbent assays, and the serum ALT assay was performed using the kinetic method within 2 h of arrival at the laboratory. The reagents used in local laboratories were tested by the National Center of Clinical Laboratories for Quality Inspection and Detection (Beijing, China), during which the reference standard was reagents produced by Abbott (Abbott Park, IL, USA). All tests provided sensitivity, specificity, and κ value > 95%.

Elevated ALT was defined as > 40 IU/L. Simultaneous seropositivity for both HBsAg and HBeAg (HBsAg+/HBeAg+) was taken to indicate ongoing infection with actively replicating virus.[30] Simultaneous seropositivity for HBsAg and seronegativity for HBeAg (HBsAg+/HBeAg–) were taken to indicate relatively lower viral load and weaker infectivity than HBsAg+/HBeAg+. Based on their serological status before pregnancy, women in the study were classified into two groups as HBsAg– or HBsAg+; or into three groups as HBsAg–, HBsAg+/HBeAg–, or HBsAg+/HBeAg+.

Classification of congenital malformations

Congenital malformations in the NPBDSN database were diagnosed prenatally at hospitals accredited for such diagnosis, or after delivery by obstetricians, pediatricians or surgeons. We extracted data on congenital malformations of the nervous system (ICD-10 code Q00–Q07); eye, ear, face and neck (Q10–Q18); cardiovascular system (Q20–Q28); respiratory system (Q30–Q34); cleft lip and cleft palate (Q35–Q37); digestive system (Q38–Q45); reproductive system (Q50–Q56); urinary system (Q60–Q64); musculoskeletal system (Q65–Q79); or "other" malformations (Q80–Q89).

Statistical analyses

Depending on whether the distribution of the continuous variables is normal or skewed, they were reported as mean and standard deviation (SD) or as median and interquartile range (IQR), while categorical variables were reported as n (%). The chi-squared test was used to examine the differences between the groups HBsAg– and HBeAg+. Missing data on maternal age at delivery, body mass index (BMI), education level, occupation, parity (primi/multi), smoking, alcohol drinking, or other microbial infections (yes/no) were addressed using multiple imputation by chained equations (MICE).[31] All variables included in the analysis were incorporated in the imputation model, including the exposure, the outcome and covariates. Nearly 13% of the subjects had missing values, so 13 sets of imputed datasets (10 iterations of each) were generated.[32]

Associations between maternal HBV infection before pregnancy and risk of congenital malformations in offspring were explored using multilevel logistic regression. County/district was set as a random intercept in order to account for clustering within a given county/district, including potential correlations in data for women within the same county/district and regional variations in how completely or accurately congenital malformations were diagnosed. The models were adjusted for the following covariates that differed significantly between HBsAg– and HBeAg+ groups: maternal age at delivery (15–53 years old), BMI at the NFPHEP physical exam (10.8–50.0 kg/m^2), parity (primipara/multipara), maternal education level (primary school or below/junior high school/senior high school/college or higher), maternal occupation (farmer/labourer/other), alcohol drinking before pregnancy (yes/no), parental smoking before pregnancy (either/neither) and other microbial infections (yes/no). Considering the potential effects of some factors reported in the literature, we also adjusted the models for the following: maternal ethnicity (Han/minority)[33,34] and presence of maternal chronic disease (defined as hypertension, diabetes mellitus, chronic nephritis, thyroid dysfunction, heart disease, and/or neoplasm).[35–38] Considering the variations in prevalence of HBV infection over time[39] and variations in prevalence of congenital malformations among months,[40] we adjusted for the year when the woman became pregnant (2010–2020) and the month when the women became pregnant (1–12). We also adjusted for the time interval between the last HBV examination and current pregnancy (≤1, 1–2, 2–3 or >3 years) because it may influence the reliability of the pre-pregnancy HBeAg status. Detailed definitions of covariates in this study could be found in Appendix S1. Variables of maternal age at delivery, year when the woman became pregnant, month when the women became pregnant, and BMI at the NFPHEP physical exam were treated as continuous data, and were transformed using cubic spline before model adjustment. Regression results were reported as odds ratios (ORs) and 95% confidence intervals (CIs).

Based on prevalence of HBsAg seropositivity reported in a national observational study[10] and results from the multilevel logistic regression described above, we calculated the population attributable fraction (PAF) of congenital malformations due to maternal HBV infection before pregnancy,[41] which indicates the incidence of congenital malformations in the population that would be eliminated if HBsAg seropositivity in pregnant women were eliminated. We also calculated the absolute risk reduction (ARR) and number needed to treat (NNT).[42,43] The NNT indicates the number of cases of HBsAg seropositivity that would need to be prevented to prevent one birth with congenital malformations. The calculation formula could be found in Appendix S2.

We repeated the logistic regression for different subgroups stratified by organ systems of congenital

malformations occurrence. Considering that women with different sociodemographic characteristics may differ in their awareness of, attitude toward, or ability to cope with HBV infection, all of which may influence the effect of HBV infection,[44,45] we tested for interactions involving maternal age group at delivery, education level, ethnicity, occupation, urban or rural residence, parental smoking before pregnancy, alcohol drinking before pregnancy and residence in HBV-endemic areas. Assuming elevated ALT may modify the association between HBV infection before pregnancy and congenital malformations in offspring, we tested for an interaction between elevated ALT and HBV infection. We also performed sensitivity analyses in which we adjusted for interactions between socioeconomic characteristics and maternal chronic diseases in order to investigate the robustness of the association between maternal infection of HBsAg before pregnancy and congenital malformations.

All analyses were performed in R 4.0.5 (R Foundation for Statistical Computing, www.r-project.org). In all statistical tests, results associated with two-tailed $P < 0.05$ and 95% CIs excluding 1.00 were deemed significant, while those with two-tailed P between 0.05 and 0.1 were considered marginally significant.

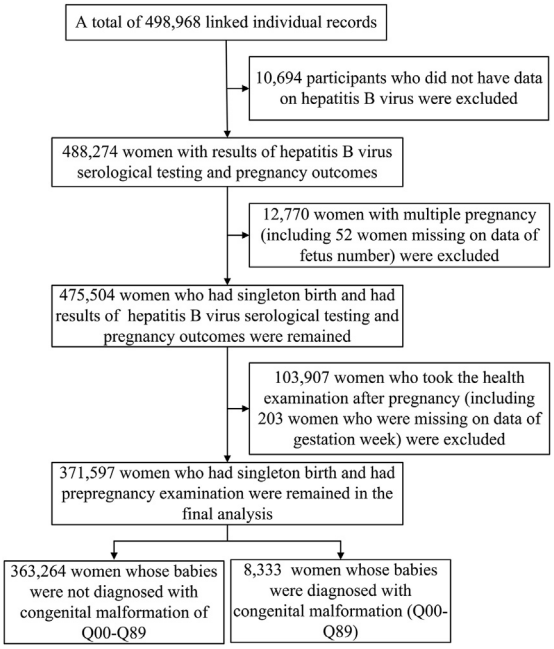

Fig. 1: **Flowchart of subject inclusion.**

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Of the 498,968 women (births) in the linked pairs of records between the two databases, we excluded 10,694 women for whom data on HBsAg or HBeAg were missing, 12,770 women whose pregnancies involved more than one fetus or an unknown number of fetuses, and 103,907 women whose blood was sampled during pregnancy or for whom the gestational week at blood sampling was unknown (Fig. 1). In the end, 371,597 women from 30 provinces across mainland China were retained in the analysis, of whom 8333 (2.24%) gave birth to babies that were diagnosed with congenital malformations.

In the final sample, 21,482 (5.78%) were HBsAg+, and among them, 15,575 (4.19%) were HBsAg+/HBeAg– and 5907 (1.59%) were HBsAg+/HBeAg+. These women, compared to those who were not infected when they were examined in the NFPHEP, were significantly more likely to be older, have a previous pregnancy and have elevated ALT (Table 1). They were also more likely to be less educated, working as farmers or labourers and infected with other microbial infections, while less likely to be smoking or drinking. Rates of HBV infection before pregnancy did not differ significantly between women belonging to the Han majority or an ethnic minority. Furthermore, women infected with HBV had slightly lower prevalence of overall chronic diseases than those without HBV infection (21.0% vs. 21.4%; Table S1).

The rate of congenital malformations was significantly higher among offspring of women who were infected with HBV (2.74%) before pregnancy than among offspring of women who were not (2.21%) (Table 1), and the risk of congenital malformations was significantly higher among HBsAg+ women than among HBsAg– women based on multilevel logistic regression (OR 1.14, 95% CI 1.03–1.25; Table 2). If this association is causal, elimination of the maternal HBsAg infection could reduce the risk of congenital malformations by an estimated of 0.86% (PAF 0.86%, 95% CI 0.18, 1.52; Table S3). In order to prevent one child from born with congenital malformations, 331.53 cases of HBsAg seropositive before pregnancy would need to be prevented (NNT 331.53, 95% CI 186.11, 1543.41; Table S3). Subgroup analysis based on different systems of congenital malformations identified elevated risk in the case of cardiovascular malformations (OR 1.18, 95% CI 1.03–1.35) but not in other systems of malformations. Similar results were obtained after we excluded all babies with congenital malformations in multiple organ systems (Table S4): HBsAg+ status in the mother before pregnancy was associated with an increased risk of malformations in aggregate (OR 1.13, 95% CI 1.02–1.25) and malformations affecting the cardiovascular system (OR 1.17, 95% CI 1.01–1.35).

	All women	HBsAg−	HBsAg+		
			Total	HBeAg−	HBeAg+
Total, n (%)	371,597 (100.00)	350,115 (94.22)	21,482 (5.78)	15,575 (4.19)	5907 (1.59)
Maternal age, yr					
Median (IQR)	29 (26–31)	29 (26–31)	29 (27–32)	29 (27–32)	28 (26–31)
<35, n (%)	335,878 (90.39)	31,6901 (90.51)	18,977 (88.34)	13,501 (86.68)	5476 (92.70)
35+, n (%)	35,705 (9.61)	33,201 (9.48)	2504 (11.66)	2074 (13.32)	430 (7.28)
Unknown	14 (0.00)	13 (0.01)	1 (0.00)	0 (0.00)	1 (0.02)
Education level					
Primary or below	15,819 (4.26)	14,620 (4.18)	1199 (5.58)	773 (4.96)	426 (7.21)
Junior high school	131,021 (35.26)	122,882 (35.10)	8139 (37.89)	5648 (36.26)	2491 (42.17)
Senior high school	73,193 (19.70)	68,842 (19.66)	4351 (20.25)	3139 (20.15)	1212 (20.52)
College or higher	124,897 (33.61)	119,019 (33.99)	5878 (27.36)	4559 (29.27)	1319 (22.33)
Unknown	2,6667 (7.18)	24,752 (7.07)	1915 (8.91)	1456 (9.35)	459 (7.77)
Occupation[a]					
Farmer	179,929 (48.42)	168,973 (48.26)	10,956 (51.00)	7615 (48.89)	3341 (56.56)
Labourer	26,611 (7.16)	24,806 (7.09)	1805 (8.40)	1359 (8.73)	446 (7.55)
Other	134,847 (36.29)	128,310 (36.65)	6537 (30.43)	4947 (31.76)	1590 (26.92)
Unknown	30,210 (8.13)	28,026 (8.00)	2184 (10.17)	1654 (10.62)	530 (8.97)
Ethnic origin					
Han	342,337 (92.13)	322,554 (92.13)	19,783 (92.09)	14,416 (92.56)	5367 (90.86)
Minority	29,260 (7.87)	27,561 (7.87)	1699 (7.91)	1159 (7.44)	540 (9.14)
Parity					
Primipara	163,270 (43.94)	155,678 (44.46)	7592 (35.34)	5494 (35.27)	2098 (35.52)
Multipara	208,270 (56.05)	194,383 (55.52)	13,887 (64.64)	10,078 (64.71)	3809 (64.48)
Unknown	57 (0.02)	54 (0.02)	3 (0.01)	3 (0.02)	0 (0.00)
Body mass index, kg/m^2					
Median (IQR)	20.55 (19.05–22.49)	20.55 (19.07–22.50)	20.31 (18.82–22.19)	20.34 (18.87–22.23)	20.03 (18.67–22.03)
<18.5	64,752 (17.43)	60,465 (17.27)	4287 (19.96)	2955 (18.97)	1332 (22.55)
18.5–23.9	251,607 (67.71)	237,353 (67.79)	14,254 (66.35)	10,421 (66.91)	3833 (64.89)
≥24.0	50,331 (13.54)	47,872 (13.67)	2459 (11.45)	1863 (11.96)	596 (10.09)
Unknown	4907 (1.32)	4425 (1.26)	482 (2.24)	336 (2.16)	146 (2.47)
Alanine aminotransferase activity, IU/L					
≤40	343,490 (92.44)	325,386 (92.94)	18,104 (84.28)	13,863 (89.01)	4241 (71.80)
>40	21,823 (5.87)	18,733 (5.35)	3090 (14.38)	1497 (9.61)	1593 (26.97)
Unknown	6284 (1.69)	5996 (1.71)	288 (1.34)	215 (1.38)	73 (1.24)
Smoking before pregnancy					
No	287,199 (77.29)	270,300 (77.20)	16,899 (78.67)	12,130 (77.88)	4769 (80.73)
Yes	79,529 (21.40)	7,5426 (21.54)	4103 (19.10)	3114 (19.99)	989 (16.74)
Unknown	4869 (1.31)	4389 (1.25)	480 (2.23)	331 (2.13)	149 (2.52)
Alcohol drinking before pregnancy					
No	340,257 (91.57)	320,542 (91.55)	19,715 (91.77)	14,282 (91.70)	5433 (91.98)
Yes	26,350 (7.09)	25,061 (7.15)	1289 (6.00)	966 (6.21)	323 (5.47)
Unknown	4990 (1.34)	4512 (1.29)	478 (2.23)	327 (2.10)	151 (2.56)
Other microbial infections					
No	45,247 (12.18)	43,373 (12.39)	1874 (8.72)	1321 (8.48)	553 (9.36)
Yes	315,083 (84.79)	296,366 (84.65)	18,717 (87.13)	13,417 (86.14)	5300 (89.72)
Unknown	11,267 (3.03)	10,376 (2.96)	891 (4.15)	837 (5,37)	54 (0.91)
Offspring with congenital malformation(s)					
Yes	8333 (2.24)	7744 (2.21)	589 (2.74)	434 (2.79)	155 (2.62)
No	363,264 (97.76)	342,371 (97.79)	20,893 (97.26)	15,141 (97.21)	5752 (97.38)

Values are median (interquartile range, IQR) or n (%). The frequencies of all maternal characteristics, except ethnic origin, differed significantly ($P < 0.05$) between the two serological groups HBsAg− and HBsAg+. [a]"Other" included self-employed, housewife, teacher or civil servant.

Table 1: **Characteristics of women in the study, stratified by prepregnancy serological status.**

Organ specific malformations	n (%) among		Odds ratio (95% confidence interval)	
	HBsAg− women	HBsAg+ women	Crude	Adjusted[a]
All systems combined	7744 (2.21)	589 (2.74)	1.25 (1.14, 1.36)	1.14 (1.03, 1.25)
Nervous system	184 (0.05)	15 (0.07)	1.34 (0.79, 2.26)	1.06 (0.57, 1.96)
Eye, ear, face and neck	957 (0.27)	61 (0.28)	1.04 (0.81, 1.35)	0.96 (0.71, 1.28)
Cardiovascular system	3695 (1.06)	278 (1.29)	1.23 (1.09, 1.39)	1.18 (1.03, 1.35)
Cleft lip and cleft palate	307 (0.09)	23 (0.11)	1.23 (0.80, 1.88)	1.13 (0.71, 1.81)
Digestive system	228 (0.07)	16 (0.07)	1.15 (0.69, 1.91)	1.02 (0.58, 1.80)
Reproductive system	383 (0.11)	40 (0.19)	1.71 (1.24, 2.37)	1.31 (0.92, 1.88)
Urinary system	381 (0.11)	27 (0.13)	1.16 (0.79, 1.72)	1.03 (0.66, 1.59)
Musculoskeletal system	1598 (0.46)	134 (0.62)	1.37 (1.15, 1.64)	1.20 (0.99, 1.46)
Respiratory system	57 (0.02)	8 (0.04)	2.30 (1.10, 4.82)	1.44 (0.57, 3.63)
Other (ICD-10 codes Q80–Q89)	328 (0.09)	22 (0.10)	1.10 (0.71, 1.69)	1.10 (0.69, 1.74)

[a]The models were adjusted for maternal age at delivery, maternal ethnicity, BMI at the NFPHEP physical exam, parity, maternal education level, maternal occupation, alcohol drinking before pregnancy, parental smoking before pregnancy and other microbial infections, presence of maternal chronic disease, the year when the woman became pregnant, the month when the women became pregnant, and the time interval between the last HBV examination and current pregnancy, and county/district was set as a random intercept.

Table 2: **Associations between maternal infection with hepatitis B virus before pregnancy and risk of congenital malformation in offspring.**

Risk of congenital malformations in offspring was higher in women who were HBsAg+/HBeAg− (OR 1.15, 95% CI 1.03–1.28), but not in those who were HBsAg+/HBeAg+ (OR 1.12, 95% CI 0.94–1.33), than in women who were HBsAg− before pregnancy (Table 3). Subgroup analysis by systems of congenital malformations confirmed these results for malformations involving the reproductive (OR 1.51, 95% CI 1.02–2.23) or the cardiovascular system (OR 1.19, 95% CI 1.01–1.39).

Among women who were HBsAg+, elevated ALT significantly modified the association between maternal HBV infection before pregnancy and risk of congenital malformations ($P_{interaction}$ = 0.015; Fig. 2). When the analysis was limited to the subgroups of cardiovascular malformations, elevated ALT remained a modifying factor ($P_{interaction}$ = 0.048; Figure S3), and working as farmers or labourers (OR 1.38, 95% CI 1.04–1.83; $P_{interaction}$ = 0.036; Figure S3), or mothers who consumed alcohol (OR 2.21, 95% CI 1.08–4.54; $P_{interaction}$ = 0.068; Figure S3) appeared to be a modifying factor of the effect of HBsAg+/HBeAg+.

The results from models that adjusted for interactions between socioeconomic characteristics and maternal chronic diseases were consistent with those from models that did not adjust for such interactions (Table S5), suggesting robust effects of HBV infection.

Organ specific malformations	HBsAg−	HBsAg+/HBeAg−			HBsAg+/HBeAg+		
	n (%)	n (%)	Crude OR (95% CI)	Adjusted OR[a] (95% CI)	n (%)	Crude OR (95% CI)	Adjusted OR[a] (95% CI)
All systems combined	7744 (2.21)	434 (2.79)	1.27 (1.15, 1.40)	1.15 (1.03, 1.28)	155 (2.62)	1.19 (1.01, 1.40)	1.12 (0.94, 1.33)
Nervous system	184 (0.05)	12 (0.08)	1.47 (0.82, 2.64)	1.08 (0.53, 2.21)	3 (0.05)	0.97 (0.31, 3.04)	0.99 (0.32, 3.12)
Eye, ear, face and neck	957 (0.27)	46 (0.30)	1.09 (0.81, 1.46)	0.99 (0.71, 1.39)	15 (0.25)	0.93 (0.56, 1.55)	0.88 (0.51, 1.52)
Cardiovascular system	3695 (1.06)	207 (1.33)	1.27 (1.10, 1.46)	1.19 (1.01, 1.39)	71 (1.20)	1.14 (0.90, 1.45)	1.16 (0.90, 1.49)
Cleft lip and cleft palate	307 (0.09)	17 (0.11)	1.25 (0.77, 2.04)	1.18 (0.69, 2.03)	6 (0.10)	1.16 (0.52, 2.61)	1.02 (0.42, 2.49)
Digestive system	228 (0.07)	12 (0.08)	1.19 (0.67, 2.13)	1.10 (0.58, 2.09)	4 (0.07)	1.04 (0.39, 2.81)	0.83 (0.27, 2.62)
Reproductive system	383 (0.11)	33 (0.21)	1.95 (1.36, 2.78)	1.51 (1.02, 2.23)	7 (0.12)	1.09 (0.51, 2.30)	0.82 (0.36, 1.84)
Urinary system	381 (0.11)	19 (0.12)	1.13 (0.71, 1.79)	0.96 (0.57, 1.63)	8 (0.14)	1.25 (0.62, 2.52)	1.18 (0.55, 2.50)
Musculoskeletal system	1598 (0.46)	95 (0.61)	1.34 (1.09, 1.65)	1.20 (0.95, 1.50)	39 (0.66)	1.45 (1.06, 2.00)	1.22 (0.86, 1.73)
Respiratory system	57 (0.02)	8 (0.05)	3.17 (1.51, 6.65)	2.01 (0.79, 5.08)	0 (0.0)	–	–
Other (ICD-10 codes Q80–Q89)	328 (0.09)	18 (0.12)	1.24 (0.77, 1.99)	1.18 (0.71, 1.97)	4 (0.07)	0.73 (0.27, 1.94)	0.99 (0.32, 3.12)

[a]The models were adjusted for maternal age at delivery, maternal ethnicity, BMI at the NFPHEP physical exam, parity, maternal education level, maternal occupation, alcohol drinking before pregnancy, parental smoking before pregnancy and other microbial infections, presence of maternal chronic disease, the year when the woman became pregnant, the month when the women became pregnant, and the time interval between the last HBV examination and current pregnancy, and county/district was set as a random intercept.

Table 3: **Comparison of risk of congenital malformations in offspring between women who, before pregnancy, were seropositive for HBsAg and either seropositive or -negative for HBeAg.**

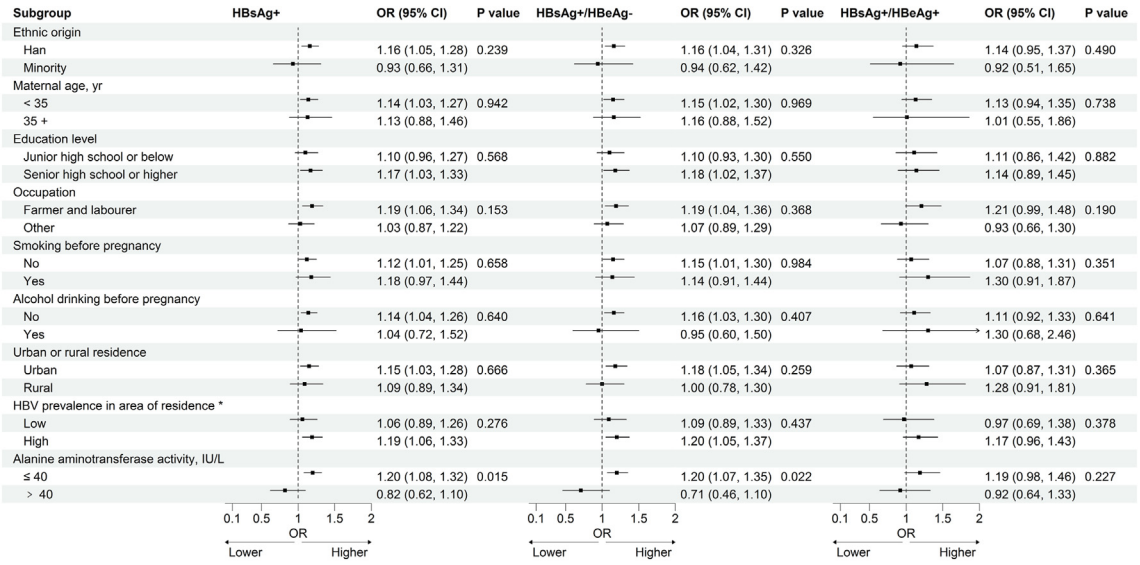

Fig. 2: **Identification of pregnancy characteristics of women that significantly influenced the association between maternal HBV infection before pregnancy and risk of congenital malformations in offspring.** *Maternal residence was classified as lying within areas of low or high HBV prevalence depending on whether the rate of seropositivity for HBsAg among pregnant women exceeded the national average of 4.8.

Discussion

Our analysis of a large national sample of women in China who planning to conceive found a prevalence of HBV infection before pregnancy of 5.78%. We also found that such infection was significantly associated with an increased risk of congenital malformations affecting certain systems in the offspring, such as cardiovascular and reproductive systems. While this phenomenon may apply only to women who are HBeAg–. Subgroup analysis showed that the effect of HBV infection may be modified by ALT levels, alcohol consumption and occupations of the mother.

Our results are consistent with previous studies reporting a link between maternal HBV infection and greater risk of congenital malformations,[23–25] but inconsistent with other studies that found no significant association.[46,47] One explanation for the apparent discrepancy is that previous studies have involved relatively small numbers of HBV-infected. Another potential explanation is strong underreporting of congenital malformations; for example, one study reported birth prevalence of congenital heart defects below 0.1%,[47] which was far lower than that of 0.5%–1.0% in the previous similar research reports.[48,49] In contrast, we found an increased risk of cardiovascular malformations in offspring from women with HBsAg+ before pregnancy.

The pathogenesis and etiology of congenital malformations attributed to maternal HBV infection remain poorly understood. Such malformations may be *direct effect* of infection on the embryo or fetus, or they may be *indirect effects* on the embryo or fetus resulting from an increase in risk of another factor contributing to fetal malformations. Direct effects may arise through germline infection, in which HBV DNA can enter oocytes, integrate into their chromosomes, and be transmitted to embryos during fertilization[21,50]; paracellular infection arising through transplacental leakage early in pregnancy due to placental immaturity, or during invasive procedures such as chorionic sampling and amniocentesis[51]; or direct infection of placental tissue.[19,52] Indirect effects may arise when, for example, HBV infection suppresses maternal immune responses to teratogenic viruses such as rubella, which can then move vertically from mother to fetus[53] or when HBV infection increases the risk of gestational diabetes mellitus in the mother,[54] which has been linked to greater risk of congenital malformations affecting certain systems, such as malformations affecting the cardiac system.[55] HBV infection was also associated with a decreased fecundability,[56] so it may increase the likelihood of assisted reproductive techniques, which is associated with an increased risk of congenital malformations.[57] While we were unable to explore the potential influence of assisted reproductive techniques on risk of congenital malformations in our sample because, the databases that we used did not record whether pregnancies were achieved through those techniques.

In our study, status of HBsAg+/HBeAg– before pregnancy was associated with an increased risk of congenital malformations, but status of HBsAg+/HBeAg+ was not. A reasonable explanation for this result may be that women with HBsAg+/HBeAg+, because of

their higher serum ALT activity and higher level of HBV DNA,[58–60] are more likely to meet the indications of clinical treatment and more likely to receive greater attention and more intensive management (e.g. antiviral treatment), which inhibits HBV proliferation in the long term and can normalize liver biochemical indicators,[61] and which may mitigate the effects of HBV infection. Similarly, HBV-infected patients with elevated ALT may be more likely to receive greater attention and more intensive management, leading to reduced level of HBV DNA, which may explain why the effect of HBV infection before pregnancy was significant among women with normal ALT before pregnancy, but not among those with elevated ALT in our study. Moreover, we found that the effect of HBV infection in HBsAg+/HBeAg+ mothers was stronger among those who consumed alcohol or worked as farmers or labourers. Alcohol consumption is a causal factor in more than 200 non communicable diseases such as liver damage[62] and in congenital malformations,[63] and it may aggravate the effect of HBV infection. However, it should be noted that data on alcohol drinking as well as smoking used in the study were obtained before pregnancy, as more than 50% of subjects in the integrated database have no data on alcohol drinking and smoking during pregnancy. Most farmers and labourers engage in manual labor, which is associated with higher burden of liver among individuals with chronic hepatitis[64]; and most famers and labourers show relatively poor health awareness and have limited access to high-quality healthcare services.[65] These factors may aggravate the effect of HBV infection on risk of congenital malformations. Our findings imply that HBV-infected women should quit drinking to reduce the impact of HBV infection on risk of congenital malformations in their offspring. They also imply that special attention should be given to female HBV-infected farmers and labourers who are planning to become pregnant.

To our knowledge, this study, which examined congenital malformations in the aggregate and individually, is the first to comprehensively evaluate the association between maternal HBV infection and organ specific malformations in offspring. Our analysis revealed a positive association between HBsAg+ before pregnancy and congenital malformations, which may be a quite interesting finding for the development of birth defect prevention strategies. Fetal organs such as central nervous and cardiac[66,67] begin developing at the third week after fertilization, and nearly all organs have formed completely around 10 weeks after fertilization. However, women usually become aware of their pregnancy after 5 weeks[68] and their first prenatal visit, when they are typically tested for HBV infection, occurs after 5 weeks. This is already too late to begin intervention in order to prevent congenital malformations such as cardiovascular malformations, if HBV infection before pregnancy is a risk factor or teratogenic factor. The guidelines for the prevention and treatment of chronic hepatitis B in Europe,[69] American,[70] and China[61] suggest that patients with HBsAg+ should be further tested for HBV DNA to determine whether antiviral treatment is necessary, and antiviral therapy is recommended for those with HBV DNA >2000 IU/ml.[69,70] However, in HBV-infected women planning to become pregnant, the threshold of HBV DNA used for the recommendation of antiviral treatment to prevent congenital malformations in offspring is unclear. It would be valuable to analyze the dose–response relationships of HBV load before pregnancy with congenital malformations in offspring.

At the same time, our findings should be interpreted carefully in light of several limitations. Firstly, as some important variables, such as HBV load, antiviral treatment, and IVF, were not available in the study, more evidence is needed for confirmation of the causality of HBV infection before pregnancy and fetal malformations. Secondly, births delivered born prior to 28 weeks of gestation were not examined in the study, which may leads to a potential underestimation of the overall birth prevalence of congenital malformations, particularly for severe cases that may result in pregnancy termination before 28 gestational weeks.[71–73] As a result, our analysis may underestimate the true associations between maternal HBV infection and risk of congenital malformations in offspring. The magnitude of this underestimation likely differs for different systems of malformations. This underestimation may, in turn, have reduced the statistical power of some analyses, potentially masking true associations between HBV infection and congenital malformations affecting certain organ systems. Thirdly, we did not finely differentiate among all possible types of congenital malformations within each organ systems because of the small numbers of fetuses or newborns affected. Besides, it should be noted that the time interval between the most recent HBV examination and pregnancy outcome exceeded two years in one third of women in our study (Table S2), and HBeAg seropositivity can revert over longer intervals. This may have biased our results, particularly those concerning HBeAg+ women, so we attempted to minimize such interference by adjusting for the time interval in our models. Nevertheless, further studies with HBeAg testing results close to pregnancy are needed for analyzing the effect of HBeAg+ before pregnancy on fetal malformations.

The results of our study imply that universal HBsAg screening of women planning to conceive might be beneficial to identify high-risk populations. It may be necessary to enhance the accessibility of HBV DNA testing services for HBsAg-positive women, especially for those with HBeAg- or normal ALT, in order to develop an individualized management or treatment plan before pregnancy, though there is currently insufficient evidence for the individualized management of infected women who are planning to become pregnant.

In fact, health care providers should be more active in providing health care services and reproductive guidance to HBsAg+ women with certain sociodemographic characteristics, such as those who drink alcohol and those engaged in famers or labourers. Future work should explore the potentially causal relationship between maternal HBV infection and congenital malformations as well as investigate the biological mechanisms involved.

Contributors

The study was conceptualized by Yuan He, Xu Ma, Yanping Wang and Xiaohong Li. Investigation (in the form of data collection and data checking) and project administration were done by Xuelian Yuan, Jun Zhu, Ying Yang, Jihong Xu, Li Dai, Huimin Li, Zhen Liu, Jing Dong and Ke Wang. Formal analysis, methodology, visualization and software were conducted by Xuelian Yuan and Xiaohong Li. An original draft was prepared by Xuelian Yuan, Xu Ma, Yuan He and Xiaohong Li, and all authors critically reviewed the draft. Xuelian Yuan and Yuan He had full access to all the data reported in this study. Xuelian Yuan accessed and verified all the data. Xiaohong Li was responsible for the decision to submit the manuscript for publication.

Data sharing statement

Data are available on reasonable request from the corresponding author. Requests will be fulfilled in accordance with national and international guidelines on data confidentiality.

Editor note

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Declaration of interests

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.lanwpc.2024.101121.

References

1 Seeger C, Mason WS. Hepatitis B virus biology. *Microbiol Mol Biol Rev*. 2000;64(1):51–68.
2 Kwon SY, Lee CH. Epidemiology and prevention of hepatitis B virus infection. *Korean J Hepatol*. 2011;17(2):87–95.
3 Polaris Observatory C. Global prevalence, treatment, and prevention of hepatitis B virus infection in 2016: a modelling study. *Lancet Gastroenterol Hepatol*. 2018;3(6):383–403.
4 *Hepatitis B fact sheet*. World Health Organization; 2019.
5 Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. *J Viral Hepat*. 2004;11(2):97–107.
6 Sirilert S, Tongsong T. Hepatitis B virus infection in pregnancy: immunological response, natural course and pregnancy outcomes. *J Clin Med*. 2021;10(13):2926.
7 Wu S, Wang J, Guo Q, et al. Prevalence of human immunodeficiency virus, syphilis, and hepatitis B and C virus infections in pregnant women: a systematic review and meta-analysis. *Clin Microbiol Infect*. 2023;29(8):1000–1007.
8 Bigna JJ, Kenne AM, Hamroun A, et al. Gender development and hepatitis B and C infections among pregnant women in Africa: a systematic review and meta-analysis. *Infect Dis Poverty*. 2019;8(1):16.
9 Organization WH. Home/Health topics/Hepatitis/Hepatitis in China. https://www.who.int/china/health-topics/hepatitis. Accessed April 22, 2024.
10 Liu J, Wang X, Wang Q, et al. Hepatitis B virus infection among 90 million pregnant women in 2853 Chinese counties, 2015-2020: a national observational study. *Lancet Reg Health West Pac*. 2021;16:100267.
11 Liu J, Liang W, Jing W, Liu M. Countdown to 2030: eliminating hepatitis B disease, China. *Bull World Health Organ*. 2019;97(3):230–238.
12 Schweitzer A, Horn J, Mikolajczyk RT, Krause G, Ott JJ. Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013. *Lancet*. 2015;386(10003):1546–1555.
13 Moorthie S, Blencowe H, Darlison MW, et al. Estimating the birth prevalence and pregnancy outcomes of congenital malformations worldwide. *J Community Genet*. 2018;9(4):387–396.
14 Organization WH. Congenital disorders. https://www.who.int/health-topics/congenital-anomalies. Accessed March 5, 2024.
15 Cui AM, Cheng XY, Shao JG, et al. Maternal hepatitis B virus carrier status and pregnancy outcomes: a prospective cohort study. *BMC Pregnancy Childbirth*. 2016;16:87.
16 Liu J, Zhang S, Liu M, Wang Q, Shen H, Zhang Y. Maternal pre-pregnancy infection with hepatitis B virus and the risk of preterm birth: a population-based cohort study. *Lancet Glob Health*. 2017;5(6):e624–e632.
17 Chen Y, Ning W, Wang X, Chen Y, Wu B, Tao J. Maternal hepatitis B surface antigen carrier status and pregnancy outcome: a retrospective cohort study. *Epidemiol Infect*. 2022;150:1–22.
18 Pereira L. Congenital viral infection: traversing the uterine-placental interface. *Annu Rev Virol*. 2018;5(1):273–299.
19 Chen Y, Wang L, Xu Y, et al. Role of maternal viremia and placental infection in hepatitis B virus intrauterine transmission. *Microbes Infect*. 2013;15(5):409–415.
20 Ye F, Liu Y, Jin Y, et al. The effect of hepatitis B virus infected embryos on pregnancy outcome. *Eur J Obstet Gynecol Reprod Biol*. 2014;172:10–14.
21 Nie R, Jin L, Zhang H, Xu B, Chen W, Zhu G. Presence of hepatitis B virus in oocytes and embryos: a risk of hepatitis B virus transmission during in vitro fertilization. *Fertil Steril*. 2011;95(5):1667–1671.
22 Hu XL, Zhou XP, Qian YL, Wu GY, Ye YH, Zhu YM. The presence and expression of the hepatitis B virus in human oocytes and embryos. *Hum Reprod*. 2011;26(7):1860–1867.
23 Huang S, Wang J, Xiong Y, et al. Impact of maternal hepatitis B carrier status on congenital abnormalities: a systematic review and meta-analysis. *BMJ Open*. 2023;13(3):e066017.
24 Tan J, Huang S, He G, et al. Maternal hepatitis B surface antigen carrier status and its impact on neonatal outcomes: a cohort study of 21 947 singleton newborns in China. *J Matern Fetal Neonatal Med*. 2017;30(18):2219–2224.
25 Wang T, Li Q, Chen L, et al. Maternal viral infection in early pregnancy and risk of congenital heart disease in offspring: a prospective cohort study in Central China. *Clin Epidemiol*. 2022;14:71–82.
26 Zhang S, Wang Q, Shen H. Design of the national free preconception health examination project in China. *Natl Med J China*. 2015;95(3):162–165.
27 Wei Y, Xu Q, Yang H, et al. Preconception diabetes mellitus and adverse pregnancy outcomes in over 6.4 million women: a population-based cohort study in China. *PLoS Med*. 2019;16(10):e1002926.
28 Dai L, Zhu J, Liang J, Wang YP, Wang H, Mao M. Birth defects surveillance in China. *World J Pediatr*. 2011;7(4):302–310.
29 von Elm E, Altman DG, Egger M, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Lancet*. 2007;370(9596):1453–1457.
30 Kao JH. Diagnosis of hepatitis B virus infection through serological and virological markers. *Expert Rev Gastroenterol Hepatol*. 2008;2(4):553–562.
31 Ware JH, Harrington D, Hunter DJ, D'Agostino RB. Missing data. *N Engl J Med*. 2012;367(14):1353–1354.

32 Buuren SV. *Flexible imputation of missing data*. 2nd ed. Chapman and Hall/CRC; 2018.
33 Chen J, Huang X, Wang B, et al. Epidemiology of birth defects based on surveillance data from 2011-2015 in Guangxi, China: comparison across five major ethnic groups. *BMC Public Health*. 2018;18(1):1008.
34 Jing W, Yuan Y, Liu M, et al. Ethnic disparities in hepatitis B virus infection among 1 million reproductive-age couples preparing for pregnancy in the rural yunnan, China: a population-based cross-sectional study. *Front Med*. 2021;8:799873.
35 Cai C, Zeng J, Wu H, et al. Association between hepatitis B virus infection and diabetes mellitus: a meta-analysis. *Exp Ther Med*. 2015;10(2):693–698.
36 Song C, Lv J, Liu Y, et al. Associations between hepatitis B virus infection and risk of all cancer types. *JAMA Netw Open*. 2019;2(6):e195718.
37 Du Y, Zhang S, Hu M, et al. Association between hepatitis B virus infection and chronic kidney disease: a cross-sectional study from 3 million population aged 20 to 49 years in rural China. *Medicine (Baltimore)*. 2019;98(5):e14262.
38 Nishiyama K, Sanefuji M, Kurokawa M, et al. Maternal chronic disease and congenital anomalies of the kidney and urinary tract in offspring: a Japanese cohort study. *Am J Kidney Dis*. 2022;80(5):619–628.e1.
39 Liu Z, Lin C, Mao X, et al. Changing prevalence of chronic hepatitis B virus infection in China between 1973 and 2021: a systematic literature review and meta-analysis of 3740 studies and 231 million people. *Gut*. 2023;72(12):2354–2363.
40 Benavides E, Lupo PJ, Langlois PH, Schraw JM. A comprehensive assessment of the associations between season of conception and birth defects, Texas, 1999-2015. *Int J Environ Res Public Health*. 2020;17(19):7120.
41 Lin CK, Chen ST. Estimation and application of population attributable fraction in ecological studies. *Environ Health*. 2019;18(1):52.
42 Ranganathan P, Pramesh CS, Aggarwal R. Common pitfalls in statistical analysis: absolute risk reduction, relative risk reduction, and number needed to treat. *Perspect Clin Res*. 2016;7(1):51–53.
43 Murad MH, Wang Z, Zhu Y, Saadi S, Chu H, Lin L. Methods for deriving risk difference (absolute risk reduction) from a meta-analysis. *BMJ*. 2023;381:e073141.
44 Gebrecherkos T, Girmay G, Lemma M, Negash M. Knowledge, attitude, and practice towards hepatitis B virus among pregnant women attending antenatal care at the university of gondar comprehensive specialized hospital, northwest Ethiopia. *Int J Hepatol*. 2020;2020:5617603.
45 Helegbe GK, Tanko F, Aryee PA, Lotsu SA, Asaarik MJA, Anaba F. High hepatitis B seroprevalence, low knowledge, and poor attitude towards hepatitis B virus infection among market women in bolgatanga metropolis in the upper east region of Ghana. *J Trop Med*. 2020;2020:4219413.
46 Luo L, Wu J, Qu Y, et al. Association between maternal HBsAg carrier status and neonatal adverse outcomes: meta-analysis. *J Matern Fetal Neonatal Med*. 2015;28(11):1308–1317.
47 Wu H, Yang Y, Jia J, et al. Maternal preconception hepatitis B virus infection and risk of congenital heart diseases in offspring among Chinese women aged 20 to 49 years. *JAMA Pediatr*. 2023;177(5):498–505.
48 Liu Y, Chen S, Zuhlke L, et al. Global birth prevalence of congenital heart defects 1970-2017: updated systematic review and meta-analysis of 260 studies. *Int J Epidemiol*. 2019;48(2):455–463.
49 Zhao L, Chen L, Yang T, et al. Birth prevalence of congenital heart disease in China, 1980-2019: a systematic review and meta-analysis of 617 studies. *Eur J Epidemiol*. 2020;35(7):631–642.
50 Huang TH, Zhang QJ, Xie QD, Zeng LP, Zeng XF. Presence and integration of HBV DNA in mouse oocytes. *World J Gastroenterol*. 2005;11(19):2869–2873.
51 Zhao X, Bai X, Xi Y. Intrauterine infection and mother-to-child transmission of hepatitis B virus: route and molecular mechanism. *Infect Drug Resist*. 2022;15:1743–1751.
52 Zhang SL, Yue YF, Bai GQ, Shi L, Jiang H. Mechanism of intrauterine infection of hepatitis B virus. *World J Gastroenterol*. 2004;10(3):437–438.
53 Lao TT, Sahota DS, Suen SS, Lau TK, Leung TY. Chronic hepatitis B virus infection and rubella susceptibility in pregnant women. *J Viral Hepat*. 2010;17(10):737–741.
54 Lao TT, Chan BC, Leung WC, Ho LF, Tse KY. Maternal hepatitis B infection and gestational diabetes mellitus. *J Hepatol*. 2007;47(1):46–50.
55 Liu Y, Yue L, Chang L. Maternal gestational diabetes mellitus and congenital heart disease in offspring: a meta-analysis. *Horm Metab Res*. 2024.
56 Zhao J, Xuan Y, Zhang Y, et al. Assessment of prior infection with hepatitis B virus and fecundability in couples planning pregnancy. *JAMA Netw Open*. 2023;6(8):e2330870.
57 Giorgione V, Parazzini F, Fesslova V, et al. Congenital heart defects in IVF/ICSI pregnancy: systematic review and meta-analysis. *Ultrasound Obstet Gynecol*. 2018;51(1):33–42.
58 Liu X, Chen JM, Lou JL, et al. Correlation between hepatitis B virus DNA levels and diagnostic tests for HBsAg, HBeAg, and PreS1-Ag in chronic hepatitis B. *Genet Mol Res*. 2016;15(2).
59 Fouad R, Musa S, Sabry D, et al. Analysis of clinical and virologic features in Hepatitis B e Antigen (HbeAg)-negative and HbeAg-positive Egyptian chronic hepatitis B patients. *Afr Health Sci*. 2020;20(2):649–655.
60 Ou XJ, Wang XM, Wang BE, et al. [An analysis of clinical features in HBeAg-negative and HBeAg-positive chronic hepatitis B]. *Zhonghua Gan Zang Bing Za Zhi*. 2007;15(6):428–430.
61 Chinese Society of Hepatology CMA, Chinese Society of Infectious Diseases CMA. Guidelines for the prevention and treatment of chronic hepatitis B (version 2022). *J Prac Hepatol*. 2023;26(3):S18–S38.
62 Organization WH. Alcohol. https://www.who.int//news-room/fact-sheets/detail/alcohol/; 2022. Accessed April 20, 2024.
63 Organization WH. Congenital disorders. https://www.who.int/news-room/fact-sheets/detail/birth-defects; 2023. Accessed April 20, 2024.
64 Kubo N, Furusyo N, Nakashima H, Kashiwagi K, Hayashi J. Strenuous physical labor is important as a cause of elevated alanine aminotransferase levels in Japanese patients with chronic hepatitis C viremia. *Eur J Epidemiol*. 2005;20(3):251–261.
65 Yuan F, Qian D, Huang C, et al. Analysis of awareness of health knowledge among rural residents in Western China. *BMC Public Health*. 2015;15:55.
66 Buijtendijk MFJ, Barnett P, van den Hoff MJB. Development of the human heart. *Am J Med Genet C Semin Med Genet*. 2020;184(1):7–22.
67 Detrait ER, George TM, Etchevers HC, Gilbert JR, Vekemans M, Speer MC. Human neural tube defects: developmental biology, epidemiology, and genetics. *Neurotoxicol Teratol*. 2005;27(3):515–524.
68 Branum AM, Ahrens KA. Trends in timing of pregnancy awareness among US women. *Matern Child Health J*. 2017;21(4):715–726.
69 European Association for the Study of the Liver. Electronic address eee, European association for the study of the L. EASL 2017 clinical practice guidelines on the management of hepatitis B virus infection. *J Hepatol*. 2017;67(2):370–398.
70 Terrault NA, Lok ASF, McMahon BJ, et al. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. *Hepatology*. 2018;67(4):1560–1599.
71 Pribadi A. Induced labour in pregnancy with congenital abnormalities: clinical indications or patient demand. *Int J Reprod Contracept Obstet Gynecol*. 2022;11(8):2301–2305.
72 Rayburn WF, Jolley JA, Simpson LL. Advances in ultrasound imaging for congenital malformations during early gestation. *Birth Defects Res A Clin Mol Teratol*. 2015;103(4):260–268.
73 Kashyap N, Pradhan M, Singh N, Yadav S. Early detection of fetal malformation, a long distance yet to cover! Present status and potential of first trimester ultrasonography in detection of fetal congenital malformation in a developing country: experience at a tertiary care centre in India. *J Pregnancy*. 2015;2015:623059.