Differences in the success of prenatal diagnosis of birth defects associated with singleton and multiple pregnancies: an observational study of more than 1.9 million births in Zhejiang Province, eastern China, during 2012–2018

Shankuan Zhu[1], Lijin Chen[1], Hong Wen[2], Haifeng Lou[2], Qingcong Kang[1], Xinning Chen[2], Danqing Chen[2], and Xiaohui Zhang[2]

[1]Zhejiang University
[2]Zhejiang University School of Medicine Women's Hospital

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Abstract

Objective: To characterize differences in the prenatal diagnosis of birth defects (BDs) associated with singleton and multiple pregnancies. Design: An observational study conducted using data from a BDs surveillance system. Setting: Eastern China. Population: A total of 54,572 babies with BDs, born in 90 hospitals located in 30 regions of Zhejiang Province between 2012 and 2018. Methods: BDs were diagnosed by obstetricians using ultra sonographic, genetic tests et al., on the basis of the ICD 10th Revision (Q00–Q99). Main outcome measures: Differences in incidence and characteristics of BDs associated with singleton and multiple pregnancies. Multivariate logistic regression models were constructed to characterize relationships between the prenatal diagnosis of BDs and multiple pregnancies, with adjustment for covariates. Results: Totals of 49,872 singletons and 3,324 multiple pregnancies with BDs were analyzed. The mean incidences of BD for single and multiple pregnancies were 26.29 and 109.99 per 1,000 births, respectively ($\chi 2$=7600, P<0.001). After adjustment for covariates, BDs associated with multiple pregnancies were less likely to be diagnosed prenatally (adjusted OR: 0.36, 95% CI: 0.32–0.40); as were congenital heart defects, congenital hydrocephalus, cleft lip with cleft palate, congenital talipes equinovarus, cleft lip without cleft palate, limb reduction defects, congenital diaphragmatic hernia, trisomy 21 syndrome, congenital malformation of the urinary system, and other chromosomal malformation, compared with singletons with BDs. Conclusions: Multiple pregnancy is associated with a significantly higher risk of BD, but a significantly lower prenatal diagnosis rate. Therefore, the healthcare of women with multiple pregnancy and their fetuses should be strengthened.

Introduction

Birth defects (BDs) are identified in approximately 1 in 300 women who undergo a routine third trimester ultrasonographic scan[1], and the global prenatal diagnosis rates vary from 15% to 86%, according to region, BD category, and the method of diagnosis.[2-5] With the rapid development of the technology for prenatal examinations, a wide range of methods have become available that improve the prenatal diagnosis of BDs. These include prenatal ultrasonography, biochemical screening, invasive techniques such as amniocentesis and chorionic villus sampling (CVS), non-invasive prenatal testing (NIPT) using cell-free fetal DNA, and gene diagnose, as well as autopsy.[6, 7]

The prevalence of multiple birth in China and internationally has been increasing, such that it is now between 0.9% and 3.3% worldwide.[8-13] This can be, at least in part, attributed to advancing maternal age and the use of assisted reproductive technologies (ARTs). The accurate prenatal diagnosis of BD associated with multiple pregnancy remains challenging, but few studies have been conducted. Club foot, Down syndrome,

and trisomy 18 have been reported to be less frequently diagnosed in twins than in singleton fetuses,[14-18] whereas Boyle *et al.* showed that multiple and singleton pregnancies were associated with comparable rates of prenatal diagnosis.[19] The methods used for prenatal diagnosis often differ for singleton and multiple pregnancies. For example, the ultrasonographic survey of general anatomy and echocardiography for the identification of congenital heart disease are recommended for twins. However, a prenatal diagnosis of aneuploidy is commonly made on the basis of the analysis of maternal serum markers, which is not specific for a particular fetus.[18,20] In addition, most previous studies were of small numbers of twins with specific malformations and used specific screening methods that can be affected by differences in technical skill level and up-to-date knowledge.

In Zhejiang, a province located in eastern China, the prevalence of twin births increased from 2.27% in 2008 to 3.01% in 2013.[11] A provincial BD surveillance system has existed there for over 30 years, and this provides a useful platform for BD-related researches. In this study, we compared the prenatal diagnosis of BDs for singleton and multiple pregnancies. We anticipate that the findings should assist the prenatal diagnosis of BDs, especially in women with multiple pregnancies.

Methods

Study design and study population

We performed an observational study using monitoring data collected as part of the BD surveillance system of Zhejiang Province, China between 2012 and 2018. This system encompassed all births, including live birth, early fetal loss, stillbirth, and neonatal deaths within 7 days of birth, that involved a BD and occurred in 90 hospitals located in the 30 regions of Zhejiang Province, which covers one-third of the total number of births in the province. A total of 54,572 births associated with a BD were recorded in the system between 2012 and 2018. All the mothers included in the system underwent routine antenatal health care visits at least five to ten times during their pregnancies, in line with the antenatal health care regulations enacted by China's Ministry of Health.[21] Questionnaire surveys regarding socio-demographic data (including maternal age, domicile, and education), obstetric characteristics (including maternal parity, singleton *vs* . multiple pregnancy, and prenatal diagnosis), and birth data (including birth weight, birth outcome, and sex) were conducted by medical staff in each of the hospitals. Quality control was performed at the local hospital and provincial levels.

For the purposes of the present study, participants for whom data were missing regarding whether they had a singleton or multiple pregnancy (n = 62), period of diagnosis (prenatal or postnatal) (n = 40), the timing of screening (in which gestational week) (n = 548), the socioeconomic status of the mother (n = 36), maternal parity (n = 524), or infant sex (n = 166) were excluded. Thus, ultimately, 53,196 births were included in the analyses.

Criteria for the diagnosis of BDs and definitions of parameters

BDs were diagnosed by obstetricians using ultrasonographic, genetic, pathologic, clinical, and laboratory tests, on the basis of the International Statistical Classification of Diseases and Related Health Problems, 10th Revision (Q00–Q99).[22] We studied 25 types of BD, including congenital heart defects (CHDs), congenital malformation of the urinary system, cleft lip with cleft palate, cleft lip without cleft palate, cleft palate without cleft lip, polydactyly, syndactyly, congenital hydrocephalus, congenital talipes equinovarus, congenital microtia, other malformation of the external ear, trisomy 21 syndrome, other chromosomal abnormalities, hypospadias, anencephaly, spina bifida, encephalocele, omphalocele, limb reduction defect, congenital atresia of the rectum and anus, congenital diaphragmatic hernia, gastroschisis, congenital esophageal atresia, conjoined twins, and exstrophy of the urinary bladder.

We categorized mothers according to their age (<20, 20–34, and [?]35 years); whether they lived in a urban or rural environment; whether their educational level was primary or below, middle or high school, or college or above; parity (0, 1, or [?]2); whether their offspring were male, female, or of unknown sex; and whether the BD of their child was diagnosed prenatally or postnatally.

Statistical analyses

Maternal age is presented as mean and standard deviation (SD). Categorical data are presented as number (N) and percentage. The incidence of BD was calculated as the number of BDs per 1,000 births. Differences in the incidence of BD and the characteristics of the BDs associated with singleton and multiple pregnancies were analyzed using ANOVA with respect to maternal age and the chi-square test for categorical variables. The chi-square test was also used to identify differences in the prenatal diagnosis rates for BDs associated with singleton and multiple pregnancies.

Multivariate logistic regression models were constructed to evaluate the relationships between the prenatal diagnosis of BDs with multiple pregnancies, after adjustment for the year of birth, maternal age group, and maternal domicile. For the BDs that were diagnosed prenatally, we further categorized the time of diagnosis as <28 or [?]28 weeks of gestation, then chi-square testing was used to identify differences in the timing of diagnosis between singleton and multiple pregnancies, considering BDs in their entirety and specific types of BD that were found to significantly differ in their prenatal diagnosis rate.

All analyses were carried out using STATA 13 (StataCorp, College Station, TX, United States).

Patient and public involvement

No patients were involved in setting the research question or the outcome measures, nor were they involved in developing plans for the design of this study or interpretation of results.

Results

Differences in the incidence and characteristics of BDs between singleton and multiple births

A total of 53,196 births between 2012 and 2018 that were associated with BDs were included in the analysis, of which 49,872 were singletons and 3,324 were multiple births (Table 1). The mean incidence of BD for multiple births was 109.99 per 1,000 births, which was significantly higher than that for singleton births (26.29 per 1,000 births; χ^2=7600, P <0.001). Significant differences were also found between singleton and multiple births associated with BDs with respect to mean maternal age, the distribution of maternal age group, maternal domicile, maternal education, maternal parity, and infant sex (all P <0.05). Compared with women who had singletons with BDs, those who had multiple pregnancies associated with BDs were more likely to be older, live in an urban environment, have a college-level education or above, and be multiparous.

Differences in the prenatal diagnosis rates of BDs between singleton and multiple pregnancies

The prenatal diagnosis rate of BDs associated with multiple pregnancies was 14.38%, which was significantly lower than that associated with singletons (32.00%; χ^2 = 452.94,P <0.001). A significantly lower prenatal diagnosis rate was found not only for BDs in their entirety associated with multiple births (χ^2 = 452.94, P <0.001), but also for 10 types of BD (all P <0.05) (Table 2). These were congenital heart defects (χ^2 = 213.89,P <0.001), congenital hydrocephalus (χ^2 = 22.05, P <0.001), cleft lip with cleft palate (χ^2 = 50.23, P <0.001), congenital talipes equinovarus (χ^2 = 5.37,P =0.020), cleft lip without cleft palate (χ^2 = 18.84, P <0.001), limb reduction defects (χ^2 = 7.74, P =0.005), congenital diaphragmatic hernia (χ^2 = 12.73, P <0.001), trisomy 21 syndrome (χ^2 = 28.40, P <0.001), congenital malformation of the urinary system (χ^2 = 29.90, P <0.001), and other chromosomal malformation (χ^2 = 4.91, P =0.027).

Relationships between the prenatal diagnosis rates for all and types of BD and multiple pregnancies

The crude and adjusted relationships between the prenatal detection rates for all and specific types of BD were summarized as odds ratios and P -values. The 10 types of BD that showed significant crude or adjusted associations are shown in Table 3. After adjustment for year of birth, maternal age group, and maternal educational level, significantly lower prenatal diagnosis rates for BDs associated with multiple births remained for BDs as a whole (adjusted odds ratio [OR_{adj}] = 0.36, 95% confidence interval [CI]: 0.32–0.40, P <0.001), congenital heart defects (OR_{adj} = 0.32, 95% CI: 0.27–0.38,P <0.001), congenital hydrocephalus (OR_{adj} = 0.24, 95% CI: 0.13–0.45,P <0.001), cleft lip with cleft palate (OR_{adj} = 0.18, 95% CI: 0.11–0.30,P <0.001),

congenital talipes equinovarus (OR_{adj} = 0.50, 95% CI: 0.27–0.92,P =0.025), cleft lip without cleft palate (OR_{adj} = 0.27, 95% CI: 0.14–0.50,P <0.001), limb reduction defects (OR_{adj} , = 0.33 95% CI: 0.16–0.68,P =0.003), congenital diaphragmatic hernia (OR_{adj} = 0.18, 95% CI: 0.06–0.58,P =0.004), trisomy 21 syndrome (OR_{adj} = 0.09, 95% CI: 0.04–0.23, P <0.001), congenital malformation of the urinary system (OR_{adj} = 0.30, 95% CI: 0.19–0.47, P <0.001), and other chromosomal malformation (OR_{adj} = 0.22, 95% CI: 0.06–0.86,P =0.030).

Differences in the timing of the prenatal diagnosis of BDs associated with singleton and multiple births

Table 4 shows the timing of the prenatal diagnosis of BDs associated with singleton and multiple births. For BDs as a whole that were associated with multiple pregnancies, 70.08% of BDs were diagnosed before 28 weeks of gestation, which was significantly lower than the 74.82% diagnosis rate for BDs associated with singletons (χ^2 = 5.51, P =0.019). The significantly lower rate of early diagnosis of BDs associated with multiple pregnancy was also found for cleft lip with cleft palate (χ^2 = 7.75,P =0.005), trisomy 21 syndrome (χ^2 = 5.46,P =0.020), and congenital malformation of the urinary system (χ^2 = 10.07, P =0.002).

Techniques used to diagnose BDs

Table 5 shows the techniques that were used to diagnose BDs. For BDs as a whole, 73.45% were diagnosed using ultrasonography, 30.02% were diagnosed using clinical tests, and 4.86% were diagnosed using chromosomal testing. Ultrasonography was the technique that was most commonly used for the identification of congenital heart defects (93.00%), congenital hydrocephalus (94.11%), cleft lip with cleft palate (86.50%), cleft lip without cleft palate (72.54%), limb reduction defects (70.93%), congenital diaphragmatic hernia (85.93%), and congenital malformation of the urinary system (93.32%). Clinical testing was the technique that was most commonly used for congenital talipes equinovarus (56.04%), and chromosomal testing was the technique that was most commonly used to diagnose trisomy 21 syndrome (85.06%) and other chromosomal malformations (79.88%).

Discussion

Main findings

Although the findings made to date regarding the incidence of BDs associated with multiple and singleton pregnancies have been inconsistent, most studies have shown that multiple pregnancies are associated with a higher risk of BD than singletons.[23, 24] However, univariate analysis alone was performed in the previous studies, and they did not categorize the BDs and had small sample sizes. In contrast, we studied multiple types of BD and the study had a large sample size of more than 1.9 million births, which should have facilitated the drawing of reliable conclusions. We found that multiple pregnancy is associated with a significantly higher risk of and a lower prenatal diagnosis rate for BDs than singleton pregnancy.

Strengths and limitations

The present study had several limitations. First, owing to limited information regarding the use of ARTs in the database, we could not adjust the data for the effect of ARTs. Several previous studies have shown that the incidence of malformations is higher in multiple pregnancies that are achieved using *in vitro* fertilization or intracytoplasmic sperm injection than in singletons. Second, most of the BDs associated with multiple pregnancies were isolated fetal abnormalities, because multiple abnormalities may lead to stillbirth or abortion.[25] Because the database does not contain information regarding spontaneous abortion, artificial abortion, or stillbirth, this may have skewed the analysis of the BD data. Thus, the high incidence of BDs associated with multiple pregnancies may have been at least in part determined by selection bias, because clinicians are more careful with respect to twin pregnancies and may therefore have conducted more diagnostic tests to check for fetal defects. These limitations should be addressed in the design of future studies.

Interpretation

Mastroiacovo and Li previously analyzed the incidence of BDs, and found that it is significantly higher in twins than in singletons.[26] In the present study, we also found that the incidence of BD associated with multiple pregnancies is more than four times higher than that associated with singletons. The previous study also showed higher incidences of a number of types of BD, such as CHD, congenital hydrocephalus, and cleft lip with or without cleft palate. Table 1 also shows that mothers of children with BDs that were derived from multiple pregnancies were more likely to be older, to live in an urban environment, to have a college education or above, and to be multiparous, than mothers who gave birth to singletons. This may be explained by the use of ARTs. Although we could not collect information regarding the use of ARTs in the present study, previous studies have shown that the age of women who are using ARTs in China is increasing, and that children born as a result of the use of some ARTs are at higher risk of BD than those that result from natural conception.[27, 28]

There was also a significantly lower prenatal diagnosis rate for BDs that were associated with multiple pregnancies than for those that were associated with singletons, and this applied to 10 specific types of BD. Eight of these were structural malformations that are normally identified in the second or third trimester. Shielding of one fetus by another in multiple pregnancies might account for this lower prenatal diagnosis rate. The study by Razavi *et al* . yielded consistent results, because they found that the accuracies of the prenatal diagnosis of congenital clubfoot in fetuses that were singletons or part of multiple pregnancies were 68.8% and 35%, respectively.

With regard to chromosomal malformations, the accuracy of screening was also lower for multiple pregnancies, despite the government of Zhejiang Province providing free serological tests as part of prenatal healthcare during the first or second trimester. At present, nuchal translucency thickness (NT) in combination with NIPT is the most effective non-invasive method of screening for Down syndrome in multiple pregnancies,[29] but this must be performed by physicians experienced in maternal and fetal care. Invasive prenatal methods of screening for chromosomal malformations in multiple pregnancies, which include CVS or amniocentesis, should also be performed by an experienced physician, because of the difficulty and risks associated.

There are few centers in Zhejiang Province where prenatal diagnosis can be performed for multiple pregnancies, but the Prenatal Diagnosis division of the China Ultrasound Association has generated standards for the prenatal ultrasonographic diagnosis of six types of BD and trained ultrasonographers for this purpose. This probably explains why there were no significant differences in the prenatal diagnosis rates of spina bifida, encephalocele, anencephaly, gastroschisis, or omphalocele between singletons and multiple pregnancies. However, owing to the limitations of the available methods of screening, the prenatal diagnosis rates for anal atresia, microtia, and esophageal atresia are extremely low: these defects can only be diagnosed using non-specific signs, with or without magnetic resonance imaging (MRI).[30, 31] Consequently, there were no significant differences in the prenatal detection rates for these three defects between singleton and multiple pregnancies. Given the greater technical challenge in prenatally diagnosing BDs in multiple pregnancies, we suggest that women with multiple pregnancies should be cared for from as early a date as possible in institutions in which the staff are capable of performing accurate prenatal diagnosis in women with multiple pregnancies. In addition, physicians should be more cautious when discussing prenatal diagnoses for women with multiple pregnancies and choose the most accurate non-invasive prenatal screening methods to avoid misdiagnoses. Finally, multiple methods of screening should be used for multiple pregnancies, such as ultrasonography, MRI, and genetic testing, as described for a combination of neurological and cardiac defects[32] to improve the prenatal diagnosis rate for BDs.

Of all the BDs diagnosed prenatally, the proportion diagnosed before 28 weeks of gestation was significantly lower in multiple pregnancies than in singleton pregnancies, and the same finding was also made for cleft lip with cleft palate, trisomy 21 syndrome, and congenital malformation of the urinary system. Congenital urinary system malformations, such as hydronephrosis, duplication of the kidney, or genital abnormalities, normally become apparent during the second or third trimester and are identified during the third trimester.[33] However, orofacial cleft may be difficult to confirm in multiple pregnancy because of the fetal position. Therefore, women with multiple pregnancies should be informed of the limitations of prenatal ul-

trasonographic screening for structural malformations of the fetus during the first and second trimesters, and should be made aware of the importance of screening for structural malformations during the third trimester, even if such screening was negative during the first. With respect to euploidy, a previous study found no significant difference in the incidence of trisomy 21 between singletons and multiple births.[34] We speculate that the limitations of NIPT, the lack of experienced personnel in many centers, sampling error, and/or cross-contamination of samples collected during invasive prenatal diagnostic procedures may be responsible for delays in diagnoses.

The high incidence of structural malformations associated with multiple pregnancies can be explained not only by of the larger number of fetuses, but also by the existence of specific complications associated with multiple births, especially in monochorionic twins. With the development of ultrasonographic methods of examination and the increasing availability of various diagnostic techniques, increasing numbers of reports have been published regarding BDs in association with multiple pregnancies. Table 5 shows that the methods used for the prenatal diagnosis of defects in multiple pregnancies mainly comprise ultrasonography, clinical history or physical examination, genetic testing, and biochemical testing. We found that the prenatal ultrasonographic diagnosis rates for CHD and hydrocephalus were very high, and indeed, in recent years, the methods of prenatal diagnosis, such as ultrasonography, serum biochemical screening, CVS, and amniocentesis, have improved substantially. More recently, great advances have been made in NT, NIPT, and ultrasonographic screening for structural defects in early pregnancy. The improvements in these techniques, accompanied by improvements in prenatal healthcare, account for the declining incidences of BDs and chromosomal anomalies. However, we should also be aware that the prenatal diagnosis of structural malformations is more difficult in multiple pregnancies, because one fetus may obscure another. Therefore, obstetricians should inform pregnant women that the prenatal diagnosis of defects is not always highly accurate, especially in multiple pregnancies.

Conclusion

Multiple pregnancy is associated with a significantly higher risk of BD and a significantly lower prenatal diagnosis rate for BDs than singleton pregnancies. Therefore, more effort should be put into the prenatal healthcare of women with multiple pregnancies.

Disclosure of interests

The authors have declared that no competing interests exist.

Contribution to authorship

XZ and LC contributed to the design. LC conducted data analysis. LC, HW and HL drafted the first version of the manuscript. XZ and SZ supervised this work. XC, QC, DC, SZ and XZ reviewed and edited the manuscript. All authors contributed to the content and critical revision of the manuscript and agreed to submit the manuscript for publication.

Details of ethics approval

This study was approved by the Ethics Committee of the Women's Hospital, Zhejiang University School of Medicine (number: 2018KY036).

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Data sharing

The data cannot be shared publicly because we are obligated to protect the privacy of participants within the BD surveillance system of Zhejiang Province, China. Data are available from the Chronic Disease Research Institute, School of Public Health, and Women's Hospital, Zhejiang University School of Medicine (zjfb@zju.edu.cn) for researchers who meet the criteria for access to confidential data.

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