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Ambient air pollutants in the first trimester of pregnancy and the risk of birth defects: an observational study

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Abstract

Objectives As current studies on the association of air pollutants and birth defects were not fully elucidated, this study aimed to examine the effects of maternal air pollutants exposure during the 1st trimester of pregnancy on the risk of birth defects.

Design An observational study.

Participants We obtained 70,854 singletons with gestational age <20 weeks who delivered at a large maternal and child health care center in Wuhan, China.

Outcome measures Birth defects data and daily average concentrates of ambient particulate matter ≤10 μm diameter (PM_{10}), particulate matter ≤2.5 μm diameter ($PM_{2.5}$), sulfur dioxide (SO_2), and nitrogen dioxide (NO_2) were obtained. Logistic regression analysis was applied to examine the effects of maternal air pollutants exposure during 1st trimester on the risks of total birth defects, congenital heart defects (CHDs), limb defects, and orofacial clefts.

Results There were a total of 1,352 birth defects cases included in this study, with a prevalence of 19.08‰. Maternal exposed to high concentrations of PM_{10}, $PM_{2.5}$, NO_2, and SO_2 in the 1st trimester were significantly associated with elevated risks of birth defects (ORs ranged from 1.10 to 1.19). Additionally, for male fetuses, maternal exposed to high $PM_{2.5}$ concentration was associated with an elevated risk of CHDs (OR=1.29, 95% CI: 1.08, 1.54). In the cold season, the risk of birth defects was significantly increased among women exposed to $PM_{2.5}$ (OR=1.31, 95% CI: 1.15, 1.50) and NO_2 (OR=1.14, 95% CI: 1.02, 1.26).

Conclusions This study showed unfavorable effects of air pollutants exposure on the risk of birth defects. Especially, maternal $PM_{2.5}$ exposure could have an elevated risk of CHDs among male fetuses, and stronger effects of $PM_{2.5}$ and NO_2 exposure on birth defects were observed in the cold season.

Keywords: air pollutants; birth defects; congenital heart defects; limb defects; orofacial clefts; pregnancy

Strengths and limitations of this study

- This study provided the first evidence on the positive associations between maternal exposed to $PM_{2.5}$ during the 1st trimester and the risk of CHDs among male fetuses but not the female fetuses.
- We also novelly found that stronger effects of air pollutants exposure during the 1st trimester on the risk of birth defects were observed in cold season.
- The selected participants are located in a large tertiary maternal care center in Wuhan and the representative of this study was undermined.
- Maternal air pollutants exposure indoor or at other living residents including work, dining, and shopping were not included and other covariates including health behaviors and genetic factors were failed to obtain.

INTRODUCTION

Birth defects are structural or functional abnormalities occurred during embryonic development, most of them forming in the 1st trimester of pregnancy.[1] Birth defects are the leading cause of fetal death, and associated with the elevated risk of childhood mortality and reduced long-term survival.[2,3] According to the Institute for Health Metrics and Evaluation, the prevalence of congenital birth defects increased from 6.08% in 2005 to 6.29% in 2019.[4] Although the upward trends were reported in severe congenital heart defects, single ventricle, atrioventricular septal defects, and tetralogy of Fallot in Europe during 1980-2012, the prevalence of birth defects are much lower (1.0-4.1‰).[5] In China, the overall prevalence of birth defects was increased from 12.83‰ in 1986 to 15.70‰ in 2014.[6] There is a necessary to explore the potential hazard factors contributed to the high prevalence of birth defects in China based on current knowledge.

Due to rapid urbanization, China has experienced severe air pollution in recent years. Report on China's implementation of the Millennium Development Goals (2000-2015) documented that particulate matter ≤10 μm diameter (PM_{10}), sulfur dioxide (SO_2), and nitrogen dioxide (NO_2) were major air pollutants in urban areas.[7] Moreover, ambient particulate matter ≤2.5 μm diameter ($PM_{2.5}$) produced by coal combustion, industry sources, and vehicular emissions, is also one of the main air pollutants of industrialization.[8] The expanding coverage of ambient air pollutants surveillance has contributed to a flow of s1tudies on the adverse pregnancy outcome, including small for gestational age and low birth weight, preterm birth, spontaneous abortion, and stillbirth in recent years.[9-11] Moreover, there is a growing interest on the associations between ambient air pollutants and birth defects, but the results of previous studies are controversial. Most studies reported that ambient air pollutants were associated with increased risks of birth defects.[12-14] However, Parkes et al. and Dolk et al. pointed out that high concentrates of PM_{10} and NO_2 exposure was not related to birth defects.[15,16] A recent meta-analysis conducted by Ma et al. even reported a protective effect of SO_2 on atrial septal defects.[17] These inconsistent results indicate that the effects of ambient air pollutants on the risk of birth defects could be varied by cultures, ethnics, or geographical distribution. More evidence is needed to clarify the risk of birth defects derived by air pollutants during the rapid social-economical development worldwide.

As current studies on the associations of air pollutants and birth defects were not fully elucidated, this study aimed to examine the effects of air pollutants on birth defects. Furthermore, the 1st trimester of pregnancy is vital for fetal development because fetal major organs and systems are formed at this stage and the fetus is most susceptible to environmental hazards. As a result, we mainly focused on the associations between maternal exposure to air pollutants including PM_{10}, $PM_{2.5}$, SO_2, and NO_2 during the 1st trimester and the risk of birth defects in Wuhan, China.

METHODS

Study site and population

This observational study was conducted in Wuhan city. Wuhan is the capital city of Hubei Province and a megacity in Central China. Its geographical location is 29° 58′ - 31° 22′ N and 113° 41′ - 115° 05′ E. The permanent resident population of Wuhan was over 10 million. Wuhan has four distinct seasons of hot summer and cold winter, with an annual average temperature of 15.8 ℃ - 17.5 ℃.[18]

There were a total of 130,186 perinatal women with detailed home addresses who delivered at Maternal and Child Health Hospital of Hubei Province, and 98,877 of them lived in Wuhan City during the 1st trimester of pregnancy. Then we obtained 74,336 participants with distances less than 10 km from home to the nearest air surveillance station. After removing 3,333 mothers of multiple pregnancies and 149 with gestational age <20 weeks, 70,854 participants were finally included in this study.

Birth defects

Birth defect cases with gestational age ≥ 20 weeks and 0-7 days after birth including elective termination of pregnancy. According to the Implementation of National Hospital Birth Defects Surveillance of China, 23 types of common birth defects were categorized according to the 10th Revision of the International Classification of Diseases (ICD-10, Q00–Q99). The top three birth defects including congenital heart defects (CHDs) (Q20–Q28), limb defects (Q69–Q72), and orofacial clefts (Q35–Q37) were further examined by an obstetrician, pediatrician, or pediatric cardiologist.

Exposure assessment

Data of the daily concentrations of air pollutants (PM_{10}, $PM_{2.5}$, NO_2, and SO_2) and the geographical locations of the air surveillance stations were obtained from China's National Urban Air Quality Real-time Publishing Platform (http://106.37.208.233:20035). Moreover, the geographical location data was converted from the detailed home address of participants by Baidu Map. The individual air pollutants were obtained according to the nearest surveillance station within 10 km from home, which was confirmed by other studies.[15,19] The median distance from home to the nearest station was 3.50 km in this study. Participants' air pollutants exposure during the 1st trimester of pregnancy was estimated by mean levels of daily concentrations.

Statistical analysis

Logistic regression analysis was applied to evaluate the effects of air pollutants exposure in the 1st trimester on the risk of total birth defects, CHDs, limb defects, and orofacial clefts. Maternal exposures to daily average air pollutants concentration during the 1st trimester were divided by interquartile range. These models were adjusted for covariates including the year of conception (2013-2018), maternal age (<25, 25-29, 30-34, and >34 years), and gravidity (1, 2-3, >3). Stratified analyses by neonatal sex (male and female fetus) and season of conception (March to August and September to February) were applied to further explore the associations between air pollutants exposure in the 1st trimester of pregnancy and the risk of birth defects. The adjusted odds ratio (OR) and 95% confidence interval (CI) were provided in each model. Statistical analyses were performed by SAS 9.4 (SAS Institute, Inc., Cary, NC).

RESULTS

There were a total of 1,352 birth defect cases among 70,854 singletons, with a prevalence of 19.08‰ (Table 1). The prevalence of birth defects increased from 21.16‰ in 2013 to 24.08‰ in 2018. It ranks first place in subpopulations who conceived in winter (26.98‰), aged <25 years (28.10‰), or with gravidity >3 times (23.93‰). In addition, the male fetuses had a higher prevalence of birth defects than female fetuses (20.57‰ *vs* 16.80‰).

Table 1 Prevalence of birth defects among the subgroups of enrolled participants in Wuhan, China

Variables	**Birth defect cases/Total**	**Prevalence (‰)**
Year of conception		
2013	272/12,856	21.16
2014	203/11,236	18.07
2015	174/11,274	15.43
2016	227/12,261	18.51
2017	226/12,844	17.60
2018	250/10,383	24.08
Season of conception		
Spring	321/18,312	17.53
Summer	295/18,853	15.65
Autumn	287/17,045	16.84
Winter	449/16,644	26.98
Age (years)		
<25	122/4,341	28.10
25–29	632/34,412	18.37
30–34	401/23,783	16.86
>34	197/8,318	23.68
Gravidity		
1	604/35,590	16.97
2-3	372/19,552	19.03
>3	376/15,712	23.93
Neonatal sex		
Male	771/37,488	20.57
Female	560/33,337	16.80
Total	1,352/70,854	19.08

Table 2 shows the distributions of daily average ambient air pollutants concentrations in the 1st trimester among the 5 groups including non-defects, birth defects, CHDs, limb defects, and orofacial clefts. The median exposures of PM_{10}, $PM_{2.5}$, NO_2, and SO_2 during the 1st trimester were 101.78μg/m³, 61.98μg/m³, 53.64μg/m³, and 13.97μg/m³ respectively among the birth defects groups.

Table 2 Quartile concentrations (μg/m³) of exposure for ambient air pollutants among birth defects groups in 1st trimester of pregnancy

Air pollutants	Non-defects N=69,502	Birth defects N=1,352	CHDs N=265	Limb defects N=210	Orofacial clefts N=119
PM_{10}					
Min	36.86	39.08	49.82	40.76	48.85
25th	80.84	84.05	84.05	85.86	83.98
Median	102.42	101.78	99.46	105.80	96.77
75th	123.86	124.39	120.48	127.34	116.36
Max	231.49	225.91	225.91	220.82	191.37
$PM_{2.5}$					
Min	21.27	21.27	22.77	22.16	22.98
25th	39.37	45.02	44.88	42.63	41.26
Median	59.21	61.98	61.33	63.00	60.98
75th	80.20	80.73	78.81	84.66	77.38
Max	178.20	165.92	154.42	154.79	155.35
NO_2					
Min	9.71	15.22	29.033	25.78	16.8901
25th	43.40	45.70	45.4945	44.31	44.0934
Median	51.49	53.64	53.6923	53.43	53.8407
75th	59.08	59.98	59.4615	62.84	59.989
Max	93.60	93.37	92.9341	93.37	87.5824
SO_2					
Min	2.93	3.02	3.93	3.77	3.99
25th	9.07	9.36	9.43	10.55	9.46
Median	14.47	13.97	13.77	16.09	12.55
75th	23.54	23.87	23.36	25.23	21.28
Max	71.74	63.36	63.36	62.99	53.75

Abbreviations: CHDs, congenital heart defects; NO_2, nitrogen dioxide; PM_{10}, particulate matter ≤10 μm diameter; $PM_{2.5}$, particulate matter <2.5 μm diameter; SO_2, sulfur dioxide.

Table 3 presents the associations of ambient air pollutants exposure in the 1st trimester of pregnancy and the risk of birth defects. We also provided the risk of birth defects in the 2nd and 3rd trimesters, as well as the entire pregnancy in the supplementary Tables S1-S3. High concentrate exposure of air pollutants was significantly associated with an increased risk of the total birth defects, yielding adjusted ORs of 1.10, 1.19, 1.16, and 1.15 for PM_{10}, $PM_{2.5}$, NO_2, and SO_2, respectively. Moreover, similar elevated risk of CHDs was observed for maternal exposure of $PM_{2.5}$ (OR=1.19, 95%CI: 1.05, 1.35), NO_2 (OR=1.12, 95%CI: 1.00, 1.25), and SO_2 (OR=1.21, 95%CI: 1.02, 1.43), but not PM_{10} (1.07, 95% CI: 0.94, 1.22).

For male fetuses, there were significantly increased risks of birth defects among mothers exposed to PM_{10}, $PM_{2.5}$, NO_2, and SO_2 by interquartile increased concentrations during the 1st trimester (OR=1.10-1.21). Moreover, a high concentration of $PM_{2.5}$ exposure was significantly associated with an elevated risk of CHDs (OR=1.29, 95% CI: 1.08, 1.54). For female fetuses, elevated risks of birth defects (OR=1.10-1.18) were observed among maternal exposed to heavy concentrated PM_{10}, $PM_{2.5}$, NO_2, and SO_2.

After stratified by season, the results show that the risk of birth defects was significantly increased among women who conceived in Autumn/Winter and were exposed to $PM_{2.5}$ (OR=1.31, 95% CI: 1.15, 1.50) and NO_2 (OR=1.14, 95% CI: 1.02, 1.26). $PM_{2.5}$ was also positively related to the increased risk of CHDs (OR=1.44, 95%CI: 1.08, 1.93). Elevated risk of orofacial clefts was observed among women who conceived in cold season exposed to heavy NO_2 concentrations (OR=1.51, 95% CI: 1.02, 2.24). However, in the warm season, the risk of limb defects was elevated among women exposed to SO_2 (OR=1.34, 95% CI: 1.01, 1.77), but decreased risk of orofacial clefts was observed among them (OR=0.64, 95% CI: 0.44, 0.94).

Table 3 Adjusted odds ratios and 95% confidence interval of ambient air pollutants for each interquartile increase during the 1st trimester and birth defects

Birth defects [a]	**PM_{10} OR (95%CI)**	**$PM_{2.5}$ OR (95%CI)**	**NO_2 OR (95%CI)**	**SO_2 OR (95%CI)**
Total				
Birth defects	**1.10(1.03,1.17)**	**1.19(1.13,1.26)**	**1.16(1.11,1.22)**	**1.15(1.07,1.25)**
CHDs	1.07(0.94,1.22)	**1.19(1.05,1.35)**	**1.12(1.00,1.25)**	**1.21(1.02,1.43)**

Limb defects	1.07(0.92,1.23)	1.10(0.95,1.27)	1.12(0.99,1.27)	1.16(0.96,1.40)
Orofacial clefts	0.91(0.75,1.10)	1.04(0.86,1.26)	1.10(0.93,1.30)	0.93(0.73,1.20)
Male fetus				
Birth defects	**1.10(1.01,1.19)**	**1.21(1.12,1.30)**	**1.15(1.08,1.23)**	**1.16(1.05,1.29)**
CHDs	1.18(0.98,1.42)	**1.29(1.08,1.54)**	1.11(0.95,1.30)	1.19(0.94,1.50)
Limb defects	1.08(0.90,1.30)	1.07(0.90,1.28)	1.12(0.95,1.30)	1.16(0.91,1.46)
Orofacial clefts	0.88(0.69,1.13)	1.10(0.86,1.41)	1.06(0.85,1.31)	0.99(0.72,1.37)
Female fetus				
Birth defects	**1.10(1.00,1.20)**	**1.18(1.08,1.29)**	**1.18(1.09,1.28)**	**1.15(1.03,1.29)**
CHDs	0.94(0.78,1.13)	1.05(0.88,1.27)	1.11(0.95,1.31)	1.21(0.95,1.55)
Limb defects	1.06(0.83,1.35)	1.13(0.89,1.45)	1.14(0.92,1.42)	1.14(0.83,1.58)
Orofacial clefts	0.97(0.71,1.32)	0.94(0.70,1.27)	1.21(0.92,1.58)	0.84(0.57,1.23)
Autumn/Winter				
Birth defects	0.90(0.80,1.02)	**1.31(1.15,1.50)**	**1.14(1.02,1.26)**	1.04(0.90,1.20)
CHDs	0.90(0.70,1.18)	**1.44(1.08,1.93)**	1.23(0.97,1.57)	1.17(0.86,1.60)
Limb defects	1.05(0.75,1.46)	1.26(0.87,1.83)	1.26(0.94,1.68)	0.96(0.66,1.41)
Orofacial clefts	0.86(0.57,1.29)	1.36(0.86,2.13)	**1.51(1.02,2.24)**	1.29(0.79,2.09)
Summer/Spring				
Birth defects	1.08(0.98,1.20)	1.02(0.91,1.16)	1.09(1.00,1.19)	1.05(0.94,1.19)
CHDs	1.00(0.80,1.25)	0.89(0.68,1.17)	0.89(0.73,1.09)	1.07(0.82,1.39)
Limb defects	1.09(0.86,1.38)	1.11(0.83,1.48)	1.12(0.92,1.38)	**1.34(1.01,1.77)**
Orofacial clefts	0.75(0.54,1.04)	0.69(0.46,1.03)	0.89(0.66,1.19)	**0.64(0.44,0.94)**

Abbreviations: CHDs, congenital heart defects; CI, confidence interval; NO_2, nitrogen dioxide; OR, odds ratio; PM_{10}, particulate matter ≤10 μm diameter; $PM_{2.5}$, particulate matter <2.5 μm diameter; SO_2, sulfur dioxide.

[a] adjusted for year of conception, maternal age, and gravidity.

DISCUSSION

In the current study, the prevalence of birth defects was close to what was reported in Hunan Province (19.18‰) and higher than that of Jiangsu Province (7.15‰).[20,21] Moreover, this study examined the associations between the risk of birth defects and air pollutants exposure in the 1st trimester, and we further explored the relationships stratified by fetal sex and season of conception.

This study showed that maternal exposure to ambient air pollutants including PM_{10}, $PM_{2.5}$, NO_2, and SO_2 during the 1st trimester could have higher risks of birth defects, which has been well documented previously. A case-control study conducted by Al Noaimi et al. showed a positive association between $PM_{2.5}$ exposure in the 1st trimester of pregnancy and the risk of birth defects (OR=1.05).[22] Wang et al. showed that maternal exposure to PM_{10} and NO_2 in the early pregnancy significantly increased the risk of birth defects by 10.3% per 10 μg/m3 and 3.4% per 10 μg/m3, respectively.[23]

As was observed among the total participants, the risk of birth defects among both male fetuses and female fetuses was significantly related to PM_{10}, $PM_{2.5}$, NO_2, and SO_2. The underlying mechanisms between air pollutants exposure and the development of birth defects are still unclear. Maternal air pollutants exposures might cause changes in epigenetic signatures and permanent modifications in gene expression.[24] Studies based on mice models showed that maternal exposure to $PM_{2.5}$ during pregnancy leads to spatial memory dysfunction, neurodevelopmental impairment, and disturbed cerebral cortex development of mice offspring.[25,26] Another study showed that maternal exposure to a high concentrate of $PM_{2.5}$ during the 1st trimester of pregnancy could result in the decreased placental expression of *BDNF* and *SYN1*, which may undermine fetal neurodevelopment.[27] Moreover, SO_2 is a systemic toxic agent which can induce chromosomal aberrations, sister chromatid exchanges, and micronuclei in mammalian cells.[28]

Except for PM_{10}, this study demonstrated adverse associations between $PM_{2.5}$, NO_2, and SO_2 exposure and the risk of CHDs. Previous studies in Taiwan and Northeast England also reported insignificant associations between PM_{10} exposure in the 1st trimester and the risk of CHDs,[29,30] whereas others confirmed the increased risk of CHDs caused by PM_{10}.[12,23,31-34] Furthermore, Huang et al. showed that $PM_{2.5}$ exposure per each interquartile increase during gestational weeks 3-8 was related to an increased risk of CHDs (OR=1.21).[35] Additionally, the positive association between the risk of CHDs and maternal exposure to NO_2 and SO_2 was documented by studies conducted by Baldacci et al. and Vrijheid et al.[36,37] Moreover, a case-control study conducted by Jiang et al. showed that maternal exposure to SO_2 during the 1st trimester was significantly associated with increased risk of CHD (OR=1.78-2.04), and Hansen et al. also confirmed that a 0.6 ppb increase in SO_2 was associated with an increased risk of aortic artery and valve defects (OR=10.76).[19,38] The heterogeneity in these studies might be explained by the variation in the population, the gestational periods, or the measurement air pollutants exposure. The physiopathological mechanism of the associations between air pollutants and CHDs was not fully elucidated. It is hypothesized that air pollutants exposure during the 1st pregnancy might strengthen the genetically and environmental interaction on the risk of CHDs.[37] Also, Air pollutants might change the molecule of DNA sequence or alter epigenetics in related to CHDs.[39]

Interestingly, only male fetuses showed an elevated risk of CHDs who were exposed to a higher $PM_{2.5}$ concentration. This result indicates that $PM_{2.5}$ might have a stronger effect on the expression of specific CHDs genes located on the Y chromosome. A study of mice models showed that increased pathological damage in hearts of offspring mice was observed among maternal mice exposure to $PM_{2.5}$, and these effects in the male group were more obvious than in the female group. Meanwhile, this study suggested that heart damage caused by maternal exposure to $PM_{2.5}$ was worse in male mice in contrast to female mice.[40]

After stratified by season, maternal exposure of $PM_{2.5}$ and NO_2 were positively associated with the risk of birth defects in the cold season, but this relationship was not observed in the warm season. Zhao et al. reported that the effects of air pollutants on the risk of birth defects were more obvious in the warm season in Hohhot.[41] This disparity could be partly explained by the uneven levels of dwellings' air pollutants. Compared to cities with lower GDP, cities with a higher GDP and a large population might have lower concentrations of indoor particulate matter.[42] Additionally, this study reported a negative association between SO_2 exposure and the risk of orofacial clefts in the warm season, which was in contrast with the results reported previously.[38] This result might be partly explained by the hypothesis that air pollutants could impact the survival of the fetus with birth defects, rather than causing the malformation development.[43] Moreover, Wuhan is a well-known city of hot summer. Most of the residential buildings are equipped with air conditioners, which could help to improve indoor air quality in hot weather. Thus, the interpretation of results should be cautious that significant associations between air pollutants exposure and the risk of birth defects in the warm season in Wuhan.

Strengths

This study firstly provided the evidence on the elevated risk of CHDs among mothers with heavy $PM_{2.5}$ exposure during the 1st trimester. We also novelly found the increased risks of CHDs and orofacial clefts among women who conceived in the cold season exposed to high concentrations of air pollutants.

Limitations

This study has several limitations. Firstly, the selected participants are located in a large tertiary maternal care center in Wuhan and the representative of this study was undermined. Secondly, the birth defects data of this study are manually collected and checked, but mistakes and omissions are inevitable. Thirdly, maternal air pollutants exposure indoor or at other living residents including work, dining, and shopping were not included in this study. Fourthly, covariates including health behaviors and genetic factors which might interfere with the relationship between maternal air pollutants exposure and the risk of birth defects were failed to obtain. More research is needed to explore the pathogenic mechanism of air pollutants exposure during pregnancy and the associated birth defects.

CONCLUSIONS

Our study confirmed the unfavorable effects of maternal exposure to air pollutants (PM_{10}, $PM_{2.5}$, NO_2, and SO_2) on the risk of birth defects during the 1st trimester of pregnancy. We firstly provided the evidence on the positive associations between $PM_{2.5}$ exposure and the risk of CHDs among male fetuses but not the female fetuses. Moreover, stronger effects of of $PM_{2.5}$ and NO_2 exposure on the risk of birth defects were observed in the cold season in Wuhan. As a result, it should be noted for the risk of birth defects due to air pollutants, and reducing individual air pollutants exposure during the 1st trimester might help to birth defect control in the context of the rapid development all over the world.

Abbreviations

CHDs: congenital heart defects; CI: confidence interval; NO_2: nitrogen dioxide; OR: odds ratio; PM_{10}: particulate matter ≤10 μm diameter; $PM_{2.5}$: particulate matter ≤2.5 μm diameter; SO_2: sulfur dioxide.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Maternal and Child Health Hospital of Hubei Province (2021IECLW025). This study was based on the retrospective clinical data without any individual patient identifiers.

Patient consent for publication

Not applicable.

Patient and public involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

Availability of data and materials

The data used in this study are available from corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Yao Cheng and Jieyun Yin contributed equally to this manuscript. Yao Cheng collected the data, performed data analysis, and draft the manuscript. Jieyun Yin collected the data, performed data analysis, and edit the manuscript. Lijun Yang designed the study, collected the data, and performed data analysis. Man Xu, Xinfeng Lu, and Wenting Huang collected the data, interpreted the data, and reviewed the manuscript. Guoqiang Sun: designed the study, collected the data, and supervised the project. All authors gave final approval of the version to be submitted and agreed to be accountable for all aspects of the work.

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Table S1 Adjusted odds ratios and 95% confidence interval of ambient air pollutants for each interquartile increase during the 2[ed] trimester and birth defects

Birth defects [a]	PM_{10} OR (95%CI)	$PM_{2.5}$ OR (95%CI)	NO_2 OR (95%CI)	SO_2 OR (95%CI)
Total				
Birth defects	0.98(0.91,1.06)	0.95(0.89,1.02)	0.99(0.93,1.05)	**0.90(0.82,0.98)**
CHDs	0.99(0.80,1.22)	0.88(0.73,1.05)	0.94(0.79,1.11)	0.79(0.62,1.01)
Limb defects	1.02(0.87,1.20)	0.95(0.82,1.10)	1.04(0.91,1.19)	1.02(0.83,1.24)
Orofacial clefts	1.19(0.82,1.71)	**1.40(1.00,1.95)**	1.26(0.93,1.71)	1.29(0.82,2.03)
Male fetus				
Birth defects	0.98(0.89,1.09)	0.95(0.87,1.04)	0.94(0.87,1.02)	0.91(0.81,1.03)
CHDs	0.84(0.63,1.13)	**0.69(0.53,0.91)**	**0.69(0.54,0.88)**	**0.60(0.43,0.85)**
Limb defects	1.10(0.89,1.34)	1.00(0.84,1.20)	1.07(0.91,1.26)	1.10(0.86,1.40)
Orofacial clefts	1.12(0.69,1.81)	1.17(0.77,1.77)	1.22(0.83,1.82)	1.10(0.62,1.94)
Female fetus				
Birth defects	0.98(0.87,1.11)	0.96(0.87,1.07)	1.06(0.96,1.17)	0.88(0.77,1.01)
CHDs	1.16(0.86,1.57)	1.09(0.84,1.41)	1.28(1.00,1.64)	1.05(0.74,1.49)
Limb defects	0.90(0.69,1.17)	0.86(0.67,1.10)	1.00(0.80,1.26)	0.88(0.63,1.22)
Orofacial clefts	1.32(0.73,2.38)	**1.97(1.07,3.62)**	1.30(0.80,2.13)	1.72(0.78,3.76)
Summer/Spring				
Birth defects	0.99(0.89,1.11)	0.92(0.83,1.02)	1.02(0.93,1.12)	0.96(0.83,1.10)
CHDs	0.96(0.72,1.27)	0.82(0.62,1.07)	0.99(0.78,1.26)	0.73(0.51,1.04)
Limb defects	1.08(0.85,1.37)	0.88(0.71,1.11)	1.06(0.86,1.29)	0.92(0.68,1.23)
Orofacial clefts	1.19(0.66,2.13)	1.46(0.82,2.62)	1.06(0.65,1.72)	1.67(0.75,3.74)
Autumn/Winter				
Birth defects	0.96(0.86,1.07)	1.00(0.91,1.10)	0.98(0.89,1.07)	0.89(0.78,1.02)
CHDs	1.01(0.74,1.39)	0.99(0.76,1.29)	0.93(0.73,1.20)	0.97(0.67,1.41)
Limb defects	0.96(0.76,1.20)	1.00(0.82,1.22)	1.03(0.86,1.24)	1.11(0.84,1.46)
Orofacial clefts	1.20(0.75,1.94)	1.31(0.87,1.97)	1.35(0.90,2.02)	1.01(0.58,1.76)

Abbreviations: CHDs, congenital heart defects; CI, confidence interval; NO_2, nitrogen dioxide; OR, odds ratio; PM_{10}, particulate matter ≤10 μm diameter; $PM_{2.5}$, particulate matter <2.5 μm diameter; SO_2, sulfur dioxide.

[a] adjusted for year of conception, maternal age, and gravidity.

Table S2 Adjusted odds ratios and 95% confidence interval of ambient air pollutants for each interquartile increase during the 3rd trimester and birth defects

Birth defects [a]	PM_{10} OR (95%CI)	$PM_{2.5}$ OR (95%CI)	NO_2 OR (95%CI)	SO_2 OR (95%CI)
Total				
Birth defects	**0.87(0.82,0.92)**	**0.91(0.87,0.96)**	**0.92(0.87,0.96)**	**0.93(0.88,0.99)**
CHDs	0.90(0.79,1.03)	**0.88(0.78,0.99)**	0.90(0.81,1.00)	0.88(0.77,1.01)
Limb defects	0.96(0.83,1.11)	0.91(0.80,1.05)	0.96(0.85,1.09)	0.99(0.85,1.15)
Orofacial clefts	**0.79(0.65,0.95)**	0.98(0.82,1.16)	0.91(0.77,1.08)	0.91(0.74,1.12)
Male fetus				
Birth defects	**0.87(0.81,0.94)**	**0.90(0.83,0.96)**	**0.92(0.86,0.98)**	0.96(0.89,1.05)
CHDs	0.88(0.73,1.05)	**0.84(0.71,0.99)**	0.87(0.75,1.02)	**0.81(0.67,0.97)**
Limb defects	0.92(0.77,1.10)	0.90(0.76,1.06)	0.92(0.79,1.07)	1.03(0.85,1.25)
Orofacial clefts	**0.77(0.60,0.99)**	0.94(0.75,1.18)	0.91(0.74,1.13)	0.95(0.73,1.24)
Female fetus				
Birth defects	**0.87(0.79,0.95)**	0.94(0.87,1.02)	**0.92(0.85,0.99)**	**0.89(0.81,0.98)**
CHDs	0.94(0.78,1.14)	0.96(0.81,1.14)	0.95(0.81,1.11)	0.99(0.81,1.21)
Limb defects	1.01(0.78,1.29)	0.93(0.74,1.18)	1.05(0.85,1.29)	0.91(0.71,1.18)
Orofacial clefts	0.81(0.60,1.10)	1.02(0.77,1.35)	0.89(0.69,1.15)	0.86(0.63,1.19)
Summer/Spring				
Birth defects	**1.11(1.01,1.23)**	**1.29(1.18,1.42)**	**1.12(1.03,1.22)**	1.05(0.96,1.15)
CHDs	1.13(0.90,1.42)	**1.27(1.03,1.57)**	**1.21(1.00,1.45)**	0.94(0.77,1.15)
Limb defects	1.10(0.86,1.40)	0.97(0.75,1.26)	0.99(0.80,1.23)	1.01(0.81,1.26)
Orofacial clefts	0.96(0.68,1.37)	**1.39(1.02,1.91)**	1.24(0.93,1.64)	1.02(0.75,1.39)
Autumn/Winter				
Birth defects	**0.68(0.60,0.78)**	**0.78(0.70,0.87)**	**0.81(0.73,0.90)**	0.87(0.74,1.01)
CHDs	0.84(0.63,1.12)	**0.72(0.56,0.93)**	**0.70(0.56,0.87)**	0.86(0.61,1.22)
Limb defects	0.81(0.60,1.10)	0.82(0.63,1.08)	0.94(0.73,1.21)	1.01(0.70,1.48)
Orofacial clefts	**0.44(0.30,0.66)**	0.78(0.54,1.10)	**0.61(0.45,0.83)**	0.64(0.40,1.03)

Abbreviations: CHDs, congenital heart defects; CI, confidence interval; NO_2, nitrogen dioxide; OR, odds ratio; PM_{10}, particulate matter ≤10 μm diameter; $PM_{2.5}$, particulate matter <2.5 μm diameter; SO_2, sulfur dioxide.

[a] adjusted for year of conception, maternal age, and gravidity.

Table S3 Adjusted odds ratios and 95% confidence interval of ambient air pollutants for each interquartile increase during the entire pregnancy and birth defects

Birth defects [a]	PM_{10} OR (95%CI)	$PM_{2.5}$ OR (95%CI)	NO_2 OR (95%CI)	SO_2 OR (95%CI)
Total				
Birth defects	**0.85(0.75,0.97)**	0.98(0.87,1.11)	1.05(0.96,1.13)	0.91(0.80,1.05)
CHDs	0.87(0.61,1.24)	0.96(0.70,1.32)	1.01(0.82,1.26)	**0.70(0.50,1.00)**
Limb defects	0.91(0.69,1.19)	0.86(0.66,1.12)	1.11(0.93,1.31)	1.00(0.75,1.33)
Orofacial clefts	0.68(0.38,1.23)	1.18(0.66,2.12)	1.33(0.90,1.97)	1.35(0.71,2.59)
Male fetus				
Birth defects	**0.81(0.68,0.96)**	0.91(0.77,1.07)	0.99(0.89,1.10)	0.90(0.76,1.08)
CHDs	0.71(0.43,1.16)	0.78(0.50,1.22)	**0.70(0.52,0.94)**	**0.56(0.34,0.91)**
Limb defects	0.94(0.67,1.33)	0.79(0.58,1.09)	1.16(0.93,1.43)	0.97(0.68,1.38)
Orofacial clefts	0.65(0.30,1.41)	1.19(0.57,2.45)	1.37(0.82,2.28)	0.89(0.40,1.99)
Female fetus				
Birth defects	0.92(0.75,1.12)	1.11(0.92,1.34)	1.13(1.00,1.28)	0.93(0.75,1.14)
CHDs	1.08(0.65,1.78)	1.20(0.76,1.92)	**1.55(1.11,2.17)**	0.91(0.55,1.50)
Limb defects	0.84(0.53,1.34)	1.02(0.64,1.61)	1.02(0.76,1.36)	1.05(0.63,1.75)
Orofacial clefts	0.72(0.29,1.83)	1.15(0.44,3.02)	1.26(0.68,2.31)	2.89(0.96,8.74)
Autumn/Winter				
Birth defects	0.91(0.76,1.10)	1.14(0.97,1.35)	1.06(0.95,1.18)	1.07(0.88,1.30)
CHDs	0.94(0.58,1.53)	1.13(0.75,1.71)	1.21(0.92,1.60)	0.84(0.53,1.35)
Limb defects	0.96(0.64,1.44)	0.89(0.61,1.29)	1.06(0.84,1.33)	0.82(0.54,1.24)
Orofacial clefts	0.89(0.33,2.38)	1.60(0.60,4.25)	1.07(0.61,1.87)	**3.37(1.04,10.85)**
Summer/Spring				
Birth defects	**0.76(0.63,0.92)**	0.84(0.70,1.00)	1.03(0.91,1.17)	**0.82(0.67,0.99)**
CHDs	0.75(0.44,1.26)	0.77(0.47,1.27)	0.78(0.55,1.10)	0.63(0.36,1.07)
Limb defects	0.85(0.58,1.24)	0.83(0.57,1.20)	1.17(0.90,1.52)	1.21(0.81,1.82)
Orofacial clefts	0.59(0.28,1.24)	0.95(0.45,1.98)	1.58(0.90,2.76)	0.79(0.35,1.75)

Abbreviations: CHDs, congenital heart defects; CI, confidence interval; NO_2, nitrogen dioxide; OR, odds ratio; PM_{10}, particulate matter ≤10 μm diameter; $PM_{2.5}$, particulate matter <2.5 μm diameter; SO_2, sulfur dioxide.

[a] adjusted for year of conception, maternal age, and gravidity.

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1-2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	5-6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	6
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*6*
Bias	9	Describe any efforts to address potential sources of bias	-
Study size	10	Explain how the study size was arrived at	-
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5-6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6
		(*b*) Describe any methods used to examine subgroups and interactions	6
		(*c*) Explain how missing data were addressed	-
		(*d*) If applicable, explain how loss to follow-up was addressed	-
		(*e*) Describe any sensitivity analyses	-
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6-7
		(b) Give reasons for non-participation at each stage	-
		(c) Consider use of a flow diagram	-
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	6-7
		(b) Indicate number of participants with missing data for each variable of interest	-
		(c) Summarise follow-up time (eg, average and total amount)	-
Outcome data	15*	Report numbers of outcome events or summary measures over time	6-7
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	-
		(*b*) Report category boundaries when continuous variables were categorized	8
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8-9
Discussion			
Key results	18	Summarise key results with reference to study objectives	10-11
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13
Generalisability	21	Discuss the generalisability (external validity) of the study results	12-13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Ambient air pollutants in the first trimester of pregnancy and birth defects: an observational study

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Manuscripts

Ambient air pollutants in the first trimester of pregnancy and birth defects: an observational study

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Abstract

Objectives As current studies on the relationships between air pollutants exposure during the 1st trimester and birth defects were not fully elucidated, this study aimed to assess the association between selected air pollutants and birth defects.

Design An observational study.

Participants We obtained 70,854 singletons with gestational age <20 weeks who were delivered at a large maternal and child health care center in Wuhan, China.

Outcome measures Birth defects data and daily average concentration of ambient particulate matter ≤10 μm diameter (PM_{10}), particulate matter ≤2.5 μm diameter ($PM_{2.5}$), sulfur dioxide (SO_2), and nitrogen dioxide (NO_2) were obtained. Logistic regression analysis was applied to assess the association between maternal air pollutants exposure during 1st trimester and total birth defects, congenital heart defects (CHDs), limb defects, and orofacial clefts adjusted to potential covariates.

Results There were a total of 1,352 birth defect cases included in this study, with a prevalence of 19.08‰. Maternal exposed to high concentrations of PM_{10}, $PM_{2.5}$, NO_2, and SO_2 in the 1st trimester were significantly associated with elevated odds ratios of birth defects (ORs ranged from 1.13 to 1.23). Additionally, for male fetuses, maternal exposed to high $PM_{2.5}$ concentration was associated with an elevated odd of CHDs (OR=1.27, 95% CI: 1.06, 1.52). In the cold season, the odds ratios of birth defects were significantly increased among women exposed to $PM_{2.5}$ (OR=1.64, 95% CI: 1.41, 1.91), NO_2 (OR=1.22, 95% CI: 1.08, 1.38), and SO_2 (OR=1.26, 95%CI: 1.07, 1.47).

Conclusions This study showed unfavorable effects of air pollutants exposure during the 1st trimester on birth defects. Especially, the association between maternal $PM_{2.5}$ exposure and CHDs was only observed among male fetuses, and stronger effects of $PM_{2.5}$, NO_2, and SO_2 exposure on birth defects were observed in the cold season.

Keywords: air pollutants; birth defects; congenital heart defects; limb defects; orofacial clefts; pregnancy

Strengths and limitations of this study

- This study explored the associations between maternal air pollutants exposure during 1st trimester and a wild range of outcomes classified by total birth defects, CHDs, limb defects, and orofacial clefts based on a large population.
- The stratification analysis by neonatal sex and season of conception indicated the specific high-risk population, which provided critical evidence of the air quality control policies.
- The selected participants are located in a large tertiary maternal care center in Wuhan and the representative of this study was undermined.
- Maternal air pollutants exposure indoor or other living residents including work, dining, and shopping were not included and other covariates including health behaviors and genetic factors were failed to obtain.

INTRODUCTION

Birth defects are structural or functional abnormalities occurred during embryonic development, most of them forming in the 1st trimester of pregnancy.[1] Birth defects are the leading cause of fetal death and are associated with the elevated risk of childhood mortality and reduced long-term survival.[2,3] According to the Institute for Health Metrics and Evaluation, the prevalence of congenital birth defects increased from 6.08% in 2005 to 6.29% in 2019.[4] Although the upward trends were reported in severe congenital heart defects, single ventricle, atrioventricular septal defects, and tetralogy of Fallot in Europe during 1980-2012, the prevalence of birth defects are much lower (1.0-4.1‰).[5] In China, the overall prevalence of birth defects increased from 12.83‰ in 1986 to 15.70‰ in 2014.[6] There is a necessity to explore the potential hazard factors contributed to the high prevalence of birth defects in China based on current knowledge.

Due to rapid urbanization, China has experienced severe air pollution in recent years. The Report on China's implementation of the Millennium Development Goals (2000-2015) documented that particulate matter ≤10 μm diameter (PM_{10}), sulfur dioxide (SO_2), and nitrogen dioxide (NO_2) were major air pollutants in urban areas.[7] Moreover, ambient particulate matter ≤2.5 μm diameter ($PM_{2.5}$) produced by coal combustion, industry sources, and vehicular emissions, is also one of the main air pollutants of industrialization.[8] The expanding coverage of ambient air pollutants surveillance has contributed to a flow of studies on the adverse pregnancy outcome, including small for gestational age and low birth weight, preterm birth, spontaneous abortion, and stillbirth in recent years.[9-11] Moreover, there is a growing interest in the associations between ambient air pollutants and birth defects, but the results of previous studies are controversial. Most studies reported that ambient air pollutants were associated with increased risks of birth defects.[12-14] However, Parkes et al. and Dolk et al. pointed out that high concentrations of PM_{10} and NO_2 exposure were not related to birth defects.[15,16] A recent meta-analysis conducted by Ma et al. even reported a protective effect of SO_2 on atrial septal defects.[17] These inconsistent results indicate that the effects of ambient air pollutants on birth defects could be varied by culture, ethnicity, or geographical distribution. More evidence is needed to clarify the risk of birth defects derived from air pollutants during the rapid social-economical development worldwide.

As current studies on the relationships between air pollutants and birth defects were not fully elucidated, this study aimed to assess the association between selected air pollutants and birth defects. Furthermore, the 1st trimester of pregnancy is vital for fetal development because fetal major organs and systems are formed at this stage and the fetus is most susceptible to environmental hazards. As a result, we mainly focused on the associations between maternal exposure to air pollutants including PM_{10}, $PM_{2.5}$, SO_2, and NO_2 during the 1st trimester and birth defects in Wuhan, China.

METHODS

Patient and public involvement

Patients or the public were not involved in any part of the design, conduct, reporting, or dissemination plans of this study.

Study design, site and population

This observational study was conducted in Wuhan city. Wuhan is the capital city of Hubei Province and a megacity in Central China. Its geographical location is 29° 58′ - 31° 22′ N and 113° 41′ - 115° 05′ E. The permanent resident population of Wuhan was over 10 million. Wuhan has four distinct seasons of hot summer and cold winter, with an annual average temperature of 15.8 ℃ - 17.5 ℃.[18]

There were a total of 130,186 perinatal women with detailed home addresses who delivered at the Maternal and Child Health Hospital of Hubei Province, and 98,877 of them lived in Wuhan City during the 1st trimester of pregnancy. Then we obtained 74,336 participants with distances less than 10 km from home to the nearest air surveillance station. After removing 3,333 mothers of multiple pregnancies and 149 with gestational age <20 weeks, 70,854 participants were finally included in this study.

Birth defects

Birth defect cases with gestational age ≥ 20 weeks and 0-7 days after birth including elective termination of pregnancy. Based on the requirements of the Maternal and

Child Health Monitoring Manual in China and the Implementation of National Hospital Birth Defects Surveillance of China, 23 types of common birth defects were categorized according to the 10th Revision of the International Classification of Diseases (ICD-10, Q00–Q99). The top three birth defects including congenital heart defects (CHDs) (Q20–Q28), limb defects (Q69–Q72), and orofacial clefts (Q35–Q37) were further examined by trained obstetricians, pediatricians, or pediatric cardiologists based on clinical observation, physical examination, biochemical index, and image examination results. Strict quality control of the reported data was performed by the assigned county-level inspector every quarter year and further checked by the city-level inspector semiannually. Detailed descriptions of the 23 types of birth defects were provided in Supplementary Table 1.

Exposure assessment

Data on the daily concentrations of air pollutants (PM_{10}, $PM_{2.5}$, NO_2, and SO_2) and the geographical locations (longitude and latitude) of the air surveillance stations were obtained from China's National Urban Air Quality Real-time Publishing Platform (http://106.37.208.233:20035). Moreover, the geographical location data was converted from the detailed home address of participants by Baidu Map. The distance from each home address of participants to all of the air surveillance stations were calculated. Then the individual daily air pollutants data were obtained according to the nearest surveillance station from home, and we only keep participants who lived within 10km from the nearest surveillance station, which was confirmed by other studies.[15,19] The median distance from home to the nearest station was 3.50 km in this study. Participants' average air pollutants exposure during the 1st trimester of pregnancy was estimated by mean levels of daily concentrations.

Statistical analysis

Observed outcomes were classified as total birth defects, CHDs, limb defects, and orofacial clefts. Maternal exposure variables including PM_{10}, $PM_{2.5}$, NO_2, and SO_2 were divided by interquartile range based on the daily average concentration during the 1st trimester. Covariates including the year of conception (2013-2018), maternal age (<25, 25-29, 30-34, and >34 years), gravidity (1, 2-3, and >3), and urban/rural were obtained. Moreover, other covariates including per capita of cars, unemployment, per capita area of roads, per capita of medication beds were retrieved from the official website of Wuhan Bureau of Statistics (http://tjj.wuhan.gov.cn/). Logistic regression analysis was applied to assess the association between maternal air pollutants exposure during 1st trimester and birth defects adjusted for potential covariates. Stratified analyses by neonatal sex (male and female fetus) and season of conception by last menstrual period (March to August and September to February) were applied to further explore the associations between air pollutants exposure in the 1st trimester of pregnancy and birth defects. The adjusted odds ratio (OR) and 95% confidence interval (CI) were provided in each model. Statistical analyses were performed by SAS 9.4 (SAS Institute, Inc., Cary, NC).

RESULTS

There were a total of 1,352 birth defect cases among 70,854 singletons, with a prevalence of 19.08‰ (Table 1). The prevalence of birth defects increased from 21.16‰ in 2013 to 24.08‰ in 2018. It ranks first place in subpopulations who conceived in winter (26.98‰), aged <25 years (28.10‰), or with gravidity >3 times (23.93‰). In addition, the male fetuses had a higher prevalence of birth defects than female fetuses (20.57‰ *vs* 16.80‰).

Table 1 Prevalence of birth defects among the subgroups of enrolled participants in Wuhan, China

Variables	Birth defect cases	Total cases	Prevalence (‰)
Year of conception			
2013	272	12,856	21.16
2014	203	11,236	18.07
2015	174	11,274	15.43
2016	227	12,261	18.51
2017	226	12,844	17.60
2018	250	10,383	24.08
Season of conception			
Spring	321	18,312	17.53
Summer	295	18,853	15.65
Autumn	287	17,045	16.84
Winter	449	16,644	26.98
Age (years)			

<25	122	4,341	28.10
25–29	632	34,412	18.37
30–34	401	23,783	16.86
>34	197	8,318	23.68
Gravidity			
1	604	35,590	16.97
2-3	372	19,552	19.03
>3	376	15,712	23.93
Neonatal sex			
Male	771	37,488	20.57
Female	560	33,337	16.80
Total	1,352	70,854	19.08

Table 2 shows the distributions of daily average ambient air pollutants concentrations in the 1st trimester among the 5 groups including non-defects, birth defects, CHDs, limb defects, and orofacial clefts. The median exposures of PM_{10}, $PM_{2.5}$, NO_2, and SO_2 during the 1st trimester were 101.78μg/m³, 61.98μg/m³, 53.64μg/m³, and 13.97μg/m³ respectively among the birth defects groups.

Table 2 Quartile concentrations (μg/m³) of exposure for ambient air pollutants among birth defects groups in 1st trimester of pregnancy in Wuhan, China

Air pollutants	Non-defects N=69,502	Birth defects N=1,352	CHDs N=265	Limb defects N=210	Orofacial clefts N=119
PM_{10}					
Min	36.86	39.08	49.82	40.76	48.85
25th	80.84	84.05	84.05	85.86	83.98
Median	102.42	101.78	99.46	105.80	96.77
75th	123.86	124.39	120.48	127.34	116.36
Max	231.49	225.91	225.91	220.82	191.37
$PM_{2.5}$					
Min	21.27	21.27	22.77	22.16	22.98
25th	39.37	45.02	44.88	42.63	41.26
Median	59.21	61.98	61.33	63.00	60.98
75th	80.20	80.73	78.81	84.66	77.38
Max	178.20	165.92	154.42	154.79	155.35
NO_2					
Min	9.71	15.22	29.03	25.78	16.89
25th	43.40	45.70	45.49	44.31	44.09
Median	51.49	53.64	53.69	53.43	53.84
75th	59.08	59.98	59.46	62.84	59.99
Max	93.60	93.37	92.93	93.37	87.58

SO_2					
Min	2.93	3.02	3.93	3.77	3.99
25th	9.07	9.36	9.43	10.55	9.46
Median	14.47	13.97	13.77	16.09	12.55
75th	23.54	23.87	23.36	25.23	21.28
Max	71.74	63.36	63.36	62.99	53.75

Abbreviations: CHDs, congenital heart defects; NO_2, nitrogen dioxide; PM_{10}, particulate matter ≤10 μm diameter; $PM_{2.5}$, particulate matter <2.5 μm diameter; SO_2, sulfur dioxide.

Table 3 presents the associations between ambient air pollutants exposure in the 1st trimester of pregnancy and birth defects. We also provided the odds ratios of birth defects in the 2nd and 3rd trimesters, as well as the entire pregnancy in the supplementary Tables S2-S4. High concentrate exposure to air pollutants was significantly associated with increased odds of total birth defects, yielding adjusted ORs of 1.13, 1.23, 1.18, and 1.19 for PM_{10}, $PM_{2.5}$, NO_2, and SO_2, respectively. Moreover, similar elevated odds of CHDs was observed for maternal exposure of $PM_{2.5}$ (OR=1.21, 95%CI: 1.06, 1.38), NO_2 (OR=1.13, 95%CI: 1.01, 1.27), and SO_2 (OR=1.24, 95%CI: 1.03, 1.48), but not PM_{10} (1.08, 95% CI: 0.95, 1.24).

For male fetuses, there were significantly increased odds of total birth defects among mothers exposed to PM_{10}, $PM_{2.5}$, NO_2, and SO_2 by interquartile increased concentrations during the 1st trimester (OR=1.13-1.23). Moreover, a positive association between $PM_{2.5}$ exposure during the 1st trimester and CHDs was detected (OR=1.27, 95% CI: 1.06, 1.52). For female fetuses, elevated odds of total birth defects (OR=1.14-1.22) were also observed among maternal exposed to heavy concentrated PM_{10}, $PM_{2.5}$, NO_2, and SO_2.

After stratified by season, the results show that the odds of total birth defects were significantly increased among women who conceived in Autumn/Winter and were exposed to $PM_{2.5}$ (OR=1.64, 95% CI: 1.41, 1.91), NO_2 (OR=1.22, 95% CI: 1.08, 1.38), or SO_2 (OR=1.26, 95% CI: 1.07, 1.47). $PM_{2.5}$, NO_2, and SO_2 were also positively related to CHDs (ORs=1.36-1.84). Elevated hazard of orofacial clefts was observed among women who conceived in the cold season exposed to heavy NO_2 concentrations (OR=1.85, 95% CI: 1.16, 2.93). However, in the warm season, the odd of total birth defects was elevated among women exposed to PM_{10} (OR=1.12, 95% CI: 1.00, 1.24).

Table 3 Adjusted odds ratios and 95% confidence interval of ambient air pollutants for each interquartile increase during the 1st trimester and birth defects in Wuhan, China

Birth defects [a]	PM_{10} OR (95%CI)	$PM_{2.5}$ OR (95%CI)	NO_2 OR (95%CI)	SO_2 OR (95%CI)
Total				
Birth defects	**1.13(1.07,1.21)**	**1.23(1.15,1.30)**	**1.18(1.12,1.24)**	**1.19(1.10,1.30)**
CHDs	1.08(0.95,1.24)	**1.21(1.06,1.38)**	**1.13(1.01,1.27)**	**1.24(1.03,1.48)**
Limb defects	1.11(0.96,1.29)	1.09(0.93,1.26)	**1.15(1.01,1.31)**	1.16(0.95,1.43)
Orofacial clefts	0.90(0.74,1.10)	1.04(0.85,1.26)	1.10(0.93,1.31)	0.95(0.73,1.22)
Male fetus				
Birth defects	**1.13(1.04,1.22)**	**1.23(1.14,1.34)**	**1.18(1.10,1.26)**	**1.19(1.07,1.33)**
CHDs	1.16(0.96,1.40)	**1.27(1.06,1.52)**	1.11(0.95,1.30)	1.18(0.93,1.50)
Limb defects	1.10(0.91,1.33)	1.08(0.90,1.30)	1.15(0.98,1.35)	1.14(0.89,1.47)
Orofacial clefts	0.86(0.66,1.12)	1.09(0.85,1.41)	1.07(0.86,1.34)	0.99(0.71,1.37)
Female fetus				
Birth defects	**1.14(1.04,1.25)**	**1.22(1.12,1.34)**	**1.19(1.10,1.29)**	**1.21(1.07,1.37)**
CHDs	0.99(0.82,1.20)	1.11(0.91,1.34)	1.14(0.97,1.34)	1.30(1.00,1.71)
Limb defects	1.08(0.84,1.38)	1.15(0.90,1.47)	1.15(0.93,1.43)	1.18(0.84,1.66)
Orofacial clefts	0.98(0.71,1.34)	0.95(0.69,1.30)	1.16(0.88,1.52)	0.88(0.59,1.30)
Autumn/Winter				
Birth defects	1.05(0.92,1.20)	**1.64(1.41,1.91)**	**1.22(1.08,1.38)**	**1.26(1.07,1.47)**
CHDs	1.05(0.79,1.39)	**1.84(1.32,2.57)**	**1.36(1.03,1.80)**	**1.43(1.00,2.03)**
Limb defects	1.19(0.84,1.70)	1.48(0.99,2.22)	1.35(0.97,1.87)	1.06(0.70,1.61)
Orofacial clefts	0.95(0.61,1.47)	1.63(0.98,2.70)	**1.85(1.16,2.93)**	1.40(0.82,2.40)
Summer/Spring				
Birth defects	**1.12(1.00,1.24)**	1.06(0.93,1.21)	1.09(1.00,1.19)	1.12(0.98,1.28)
CHDs	0.99(0.77,1.26)	0.89(0.65,1.21)	0.88(0.71,1.08)	1.24(0.92,1.66)
Limb defects	1.18(0.92,1.51)	1.21(0.89,1.63)	1.17(0.95,1.43)	1.23(0.89,1.71)
Orofacial clefts	0.72(0.50,1.03)	0.66(0.41,1.04)	0.88(0.65,1.19)	0.72(0.47,1.10)

Bold values represent statistical significance (two-sided P<0.05).

Abbreviations: CHDs, congenital heart defects; CI, confidence interval; NO_2, nitrogen dioxide; OR, odds ratio; PM_{10}, particulate matter ≤10 μm diameter; $PM_{2.5}$, particulate matter <2.5 μm diameter; SO_2, sulfur dioxide.

[a] Adjusted for year of conception, maternal age, gravidity, urban/rural, per capita area of roads, per capita of medication beds, unemployment, and per capita of cars.

DISCUSSION

In the current study, the prevalence of birth defects was close to what was reported in Hunan Province (19.18‰) and higher than that of Jiangsu Province (7.15‰).[20,21] Moreover, this study examined the associations between birth defects and air pollutants exposure in the 1st trimester, and we further explored the relationships stratified by fetal sex and season of conception.

This study showed that maternal exposure to ambient air pollutants including PM_{10}, $PM_{2.5}$, NO_2, and SO_2 during the 1st trimester could have higher odds of birth defects, which has been well documented previously. Moreover, we have adopted peak exposure on the thresholds at the 95th percentile to derive the accumulated days of high dose exposure, which double confirmed the positive association between high dose of NO_2 exposure during the 1st trimester and birth defects (Supplementary Table 5). A case-control study conducted by Al Noaimi et al. showed a positive association between $PM_{2.5}$ exposure in the 1st trimester of pregnancy and birth defects (OR=1.05).[22] Wang et al. applied Poisson generalized additive models on the time-series data adjusted for temperature, relative humidity, season, and time trend, which showed that maternal exposure to PM_{10} and NO_2 in early pregnancy significantly increased the risk of birth defects by 10.3% per 10 μg/m^3 and 3.4% per 10 μg/m^3, respectively.[23]

As was observed among the total participants, the risk of birth defects among both male fetuses and female fetuses was significantly related to PM_{10}, $PM_{2.5}$, NO_2, and SO_2. The underlying mechanisms between air pollutants exposure and the development of birth defects are still unclear. Maternal air pollutants exposures might cause changes in epigenetic signatures and permanent modifications in gene expression.[24] Studies based on mice models showed that maternal exposure to $PM_{2.5}$ during pregnancy leads to spatial memory dysfunction, neurodevelopmental impairment, and disturbed cerebral cortex development of mice offspring.[25,26] Another study showed that maternal exposure to a high concentrate of $PM_{2.5}$ during the 1st trimester of pregnancy could result in the decreased placental expression of *BDNF* and *SYN1*, which may undermine fetal neurodevelopment.[27] Moreover, SO_2 is a systemic toxic agent which can induce chromosomal aberrations, sister chromatid exchanges, and micronuclei in mammalian cells.[28]

Except for PM_{10}, this study demonstrated adverse associations of CHDs with $PM_{2.5}$, NO_2, and SO_2 exposure. Previous studies in Taiwan and Northeast England also reported insignificant associations between PM_{10} exposure in the 1st trimester and CHDs,[29,30] whereas others confirmed the increased risk of CHDs caused by PM_{10}.[12,23,31-34] Furthermore, Huang et al. showed that $PM_{2.5}$ exposure per each interquartile increase during gestational weeks 3-8 was related to an increased risk of CHDs (OR=1.21).[35] Additionally, the positive association between CHDs and maternal exposure to NO_2 and SO_2 was documented by studies conducted by Baldacci et al. and Vrijheid et al.[36,37] Moreover, a case-control study conducted by Jiang et al. showed that maternal exposure to SO_2 during the 1st trimester was significantly associated with increased risk of CHD (OR=1.78-2.04), and Hansen et al. also confirmed that a 0.6 ppb increase in SO_2 was associated with an increased risk of aortic artery and valve defects (OR=10.76).[19,38] The heterogeneity in these studies might be explained by the variation in the population, the gestational periods, or the measurement of air pollutants exposure. The physiopathological mechanism of the associations between air pollutants and CHDs was not fully elucidated. It is hypothesized that air pollutants exposure during the 1st pregnancy might strengthen the genetic and environmental interaction on CHDs.[37] Also, Air pollutants might change the molecule of DNA sequence or alter epigenetics in related to CHDs.[39]

The epidemiological difference in CHD_S between male and female fetuses has been wildly reported.[40,41] This disparity could be explained by the sex chromosome-linked genes expression and their interactions with hormonal effects during early development.[42] Interestingly, only male fetuses showed an elevated risk of CHDs who were exposed to a higher $PM_{2.5}$ concentration. This result indicates that $PM_{2.5}$ might emphasize the disparity in the embryonic origins of sexual dimorphism. Moreover, $PM_{2.5}$ might have a stronger effect on the expression of specific CHDs genes located on the Y chromosome. A study of mice models showed that increased pathological damage in the hearts of offspring mice was observed among maternal mice exposed to $PM_{2.5}$, and these effects in the male group were more obvious than in the female group. $PM_{2.5}$ exposure in utero inhibited the expression of the *GATA4* gene in male mice, which related to the formation of CHDs.[43]

After stratified by season, maternal exposure of $PM_{2.5}$, NO_2, and SO_2 were positively associated with birth defects in the cold season, but this relationship was not observed in the warm season. Zhao et al. reported that the effects of air pollutants on birth defects were more obvious in the warm season in Hohhot.[44] This disparity could be partly explained by the uneven levels of dwellings' air pollutants. Compared to cities with lower GDP, cities with a higher GDP and a large population might have lower concentrations of indoor particulate matter.[45] Moreover, Wuhan is a well-known city with hot summer. Most of the residential buildings are equipped with air conditioners, which could help to improve indoor air quality in hot weather. Thus, the interpretation of results should be cautious that significant associations between air pollutants exposure and birth defects in the warm season in Wuhan.

Strengths

This study firstly provided evidence on the elevated risk of CHDs among mothers with heavy $PM_{2.5}$ exposure during the 1st trimester. We also found the increased risks of CHDs and orofacial clefts among women who conceived in the cold season exposed to high concentrations of air pollutants.

Limitations

This study has several limitations. Firstly, the selected participants are located in a large tertiary maternal care center in Wuhan and the representative of this study was undermined. Secondly, the birth defects data of this study are manually collected and checked, but mistakes and omissions are inevitable. Thirdly, maternal air pollutants exposure indoor or other living residents including work, dining, and shopping were not included in this study. Fourthly, other risk factors (including ethnicity, smoking, drinking, medications, drug use, and family history et al.) were failed to obtain, which might undermine the interpretation of the relationship between maternal air pollutants exposure and birth defects. More research is needed to explore the pathogenic mechanism of air pollutants exposure during pregnancy and the associated birth defects.

CONCLUSIONS

Our study confirmed the unfavorable effects of maternal exposure to air pollutants (PM_{10}, $PM_{2.5}$, NO_2, and SO_2) on birth defects during the 1st trimester of pregnancy. We firstly provided the evidence on the positive associations between $PM_{2.5}$ exposure and CHDs among male fetuses but not female fetuses. Moreover, stronger effects of $PM_{2.5}$, NO_2, and SO_2 exposure on birth defects were observed in the cold season in Wuhan. As a result, it should be noted for birth defects due to air pollutants, and reducing individual air pollutants exposure during the 1st trimester might help to birth defect control in the context of the rapid development all over the world. Moreover, the implementation of air quality protection policies on birth defect control should consider seasonal factor, especially for the cold season in Wuhan, China. Future studies of birth defects and air quality data collected by individual air pollutants monitors are promoted.

Abbreviations

CHDs: congenital heart defects; CI: confidence interval; NO_2: nitrogen dioxide; OR: odds ratio; PM_{10}: particulate matter ≤10 μm diameter; $PM_{2.5}$: particulate matter ≤2.5 μm diameter; SO_2: sulfur dioxide.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Maternal and Child Health Hospital of Hubei Province (2021IECLW025). This study was based on the retrospective clinical data without any individual patient identifiers.

Patient consent for publication

Not applicable.

Patient and public involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

Availability of data and materials

The data used in this study are available from corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Yao Cheng and Jieyun Yin contributed equally to this manuscript. Yao Cheng collected the data, performed data analysis, draft and revised the manuscript. Jieyun Yin collected the data, performed data analysis, and edit the manuscript. Lijun Yang designed the study, collected the data, and performed data analysis. Man Xu, Xinfeng Lu, and Wenting Huang collected the data, interpreted the data, and reviewed the manuscript. Guohong Dai: collected the data, interpreted the data, and revised the manuscript. Guoqiang Sun: designed the study, collected the data, and supervised the project. All authors gave final approval of the version to be submitted and agreed to be accountable for all aspects of the work.

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Table S1 The rank result of the 23 types of common birth defects

Number	Birth defect	N
21	Conjoined twins	0
13	Bladder valgus	1
1	Anencephaly	2
3	Encephalocele	3
20	Gastroschisis	3
8	Small ears (including no ears)	6
10	Esophageal atresia or stenosis	8
2	Spina bifida	12
18	Congenital diaphragmatic hernia	13
19	Omphalocele	18
12	Hypospadias	27
11	Rectoanal atresia or stricture (including anorectal atresia)	30
14	Equinovarus	41
4	Congenital hydrocephalus	45
22	Down's syndrome	79
9	Other malformations of the external ear (except for small ears and no ears)	86
5, 6, 7	Orofacial clefts	119
15, 16, 17	Limb defects	210
23	Congenital heart defects	265

Table S2 Adjusted odds ratios and 95% confidence interval of ambient air pollutants for each interquartile increase during the 2[ed] trimester and birth defects in Wuhan, China

Birth defects [a]	PM_{10} OR (95%CI)	$PM_{2.5}$ OR (95%CI)	NO_2 OR (95%CI)	SO_2 OR (95%CI)
Total				
Birth defects	1.01(0.93,1.09)	0.95(0.89,1.02)	0.99(0.93,1.06)	**0.88(0.80,0.97)**
CHDs	0.98(0.79,1.22)	0.86(0.72,1.04)	0.94(0.79,1.11)	**0.75(0.58,0.97)**
Limb defects	1.03(0.87,1.22)	0.95(0.82,1.11)	1.05(0.92,1.21)	1.01(0.83,1.25)
Orofacial clefts	1.27(0.86,1.87)	**1.42(1.01,1.99)**	1.27(0.93,1.74)	1.38(0.85,2.25)
Male fetus				
Birth defects	1.01(0.91,1.12)	0.95(0.87,1.04)	0.95(0.87,1.03)	0.90(0.79,1.02)
CHDs	0.82(0.61,1.10)	**0.69(0.52,0.91)**	**0.69(0.53,0.88)**	**0.58(0.41,0.83)**
Limb defects	1.12(0.90,1.38)	1.00(0.84,1.20)	1.09(0.92,1.29)	1.09(0.84,1.41)
Orofacial clefts	1.11(0.69,1.81)	1.20(0.79,1.82)	1.25(0.83,1.87)	1.19(0.67,2.12)
Female fetus				
Birth defects	1.01(0.89,1.14)	0.96(0.86,1.07)	1.06(0.96,1.17)	0.87(0.75,1.00)
CHDs	1.21(0.88,1.66)	1.07(0.82,1.39)	1.28(0.99,1.65)	1.00(0.69,1.44)
Limb defects	0.90(0.69,1.18)	0.87(0.68,1.12)	0.99(0.79,1.25)	0.89(0.63,1.25)
Orofacial clefts	1.64(0.80,3.36)	**2.02(1.06,3.86)**	1.31(0.79,2.18)	2.01(0.75,5.39)
Summer/Spring				
Birth defects	1.04(0.93,1.17)	0.91(0.82,1.01)	1.03(0.94,1.13)	0.92(0.79,1.06)
CHDs	0.99(0.73,1.33)	0.79(0.60,1.04)	1.01(0.78,1.29)	**0.64(0.44,0.94)**
Limb defects	1.10(0.86,1.41)	0.88(0.69,1.11)	1.06(0.87,1.29)	0.92(0.67,1.25)
Orofacial clefts	1.20(0.64,2.25)	1.47(0.81,2.67)	1.07(0.66,1.76)	1.71(0.74,3.97)
Autumn/Winter				
Birth defects	0.96(0.86,1.08)	1.00(0.91,1.10)	0.97(0.89,1.07)	0.87(0.75,1.00)
CHDs	1.02(0.74,1.40)	1.00(0.76,1.30)	0.93(0.72,1.19)	1.00(0.68,1.47)
Limb defects	0.94(0.74,1.18)	0.99(0.82,1.21)	1.05(0.87,1.28)	1.05(0.77,1.43)
Orofacial clefts	1.30(0.78,2.17)	1.34(0.88,2.03)	1.38(0.90,2.12)	1.08(0.58,2.03)

Bold values represent statistical significance (two-sided $P<0.05$).

Abbreviations: CHDs, congenital heart defects; CI, confidence interval; NO_2, nitrogen dioxide; OR, odds ratio; PM_{10}, particulate matter ≤10 μm diameter; $PM_{2.5}$, particulate matter <2.5 μm diameter; SO_2, sulfur dioxide.

[a] Adjusted for year of conception, maternal age, gravidity, urban/rural, per capita area of roads, per capita of medication beds, unemployment, and per capita of cars.

Table S3 Adjusted odds ratios and 95% confidence interval of ambient air pollutants for each interquartile increase during the 3[rd] trimester and birth defects in Wuhan, China

Birth defects [a]	PM_{10} OR (95%CI)	$PM_{2.5}$ OR (95%CI)	NO_2 OR (95%CI)	SO_2 OR (95%CI)
Total				
Birth defects	**0.86(0.81,0.92)**	**0.89(0.84,0.94)**	**0.90(0.85,0.95)**	**0.92(0.86,0.98)**
CHDs	0.90(0.79,1.03)	**0.87(0.77,0.98)**	**0.89(0.79,1.00)**	0.88(0.76,1.01)
Limb defects	0.96(0.83,1.12)	0.90(0.79,1.04)	0.97(0.85,1.10)	1.00(0.85,1.17)
Orofacial clefts	**0.78(0.64,0.95)**	0.97(0.81,1.16)	0.90(0.76,1.07)	0.89(0.72,1.10)
Male fetus				
Birth defects	**0.87(0.80,0.94)**	**0.88(0.81,0.94)**	**0.91(0.85,0.97)**	0.96(0.88,1.05)
CHDs	0.85(0.72,1.01)	0.88(0.74,1.05)	0.88(0.76,1.03)	**0.81(0.67,0.98)**
Limb defects	0.93(0.77,1.11)	0.89(0.75,1.05)	0.92(0.79,1.08)	1.03(0.85,1.25)
Orofacial clefts	**0.78(0.60,1.00)**	0.94(0.75,1.19)	0.93(0.75,1.16)	0.95(0.72,1.25)
Female fetus				
Birth defects	**0.86(0.78,0.94)**	**0.91(0.84,0.99)**	**0.89(0.83,0.96)**	**0.86(0.78,0.95)**
CHDs	0.93(0.77,1.14)	0.92(0.76,1.10)	0.91(0.77,1.07)	0.98(0.79,1.21)
Limb defects	1.01(0.79,1.30)	0.94(0.74,1.19)	1.05(0.85,1.31)	0.93(0.72,1.21)
Orofacial clefts	0.78(0.57,1.08)	0.99(0.74,1.32)	0.85(0.66,1.10)	0.79(0.56,1.11)
Summer/Spring				
Birth defects	1.04(0.94,1.16)	**1.18(1.07,1.31)**	1.06(0.98,1.16)	0.96(0.87,1.06)
CHDs	1.07(0.84,1.36)	1.16(0.91,1.46)	1.13(0.94,1.37)	0.84(0.68,1.05)
Limb defects	1.06(0.82,1.37)	0.89(0.66,1.19)	0.97(0.78,1.21)	0.94(0.74,1.19)
Orofacial clefts	0.96(0.66,1.39)	1.37(0.98,1.91)	1.22(0.91,1.64)	0.93(0.67,1.30)
Autumn/Winter				
Birth defects	**0.64(0.56,0.73)**	**0.78(0.70,0.87)**	**0.74(0.66,0.82)**	0.86(0.73,1.00)
CHDs	**0.74(0.55,0.99)**	**0.73(0.57,0.94)**	**0.65(0.52,0.82)**	0.82(0.58,1.15)
Limb defects	0.78(0.57,1.08)	0.82(0.62,1.08)	1.05(0.78,1.41)	1.07(0.73,1.58)
Orofacial clefts	0.78(0.55,1.10)	**0.40(0.27,0.60)**	**0.52(0.38,0.70)**	**0.62(0.39,0.99)**

Bold values represent statistical significance (two-sided $P<0.05$).

Abbreviations: CHDs, congenital heart defects; CI, confidence interval; NO_2, nitrogen dioxide; OR, odds ratio; PM_{10}, particulate matter ≤10 μm diameter; $PM_{2.5}$, particulate matter <2.5 μm diameter; SO_2, sulfur dioxide.

[a] Adjusted for year of conception, maternal age, gravidity, urban/rural, per capita area of roads, per capita of medication beds, unemployment, and per capita of cars.

Table S4 Adjusted odds ratios and 95% confidence interval of ambient air pollutants for each interquartile increase during the entire pregnancy and birth defects in Wuhan, China

Birth defects [a]	PM_{10} OR (95%CI)	$PM_{2.5}$ OR (95%CI)	NO_2 OR (95%CI)	SO_2 OR (95%CI)
Total				
Birth defects	**0.85(0.74,0.97)**	0.95(0.83,1.09)	1.04(0.95,1.13)	**0.84(0.72,0.98)**
CHDs	0.91(0.64,1.29)	0.86(0.60,1.23)	1.01(0.80,1.27)	**0.60(0.41,0.89)**
Limb defects	0.91(0.69,1.21)	0.85(0.64,1.13)	1.14(0.95,1.37)	0.97(0.71,1.34)
Orofacial clefts	0.68(0.36,1.26)	1.16(0.62,2.17)	1.34(0.89,2.04)	1.45(0.70,2.98)
Male fetus				
Birth defects	**0.81(0.68,0.97)**	0.89(0.75,1.06)	0.99(0.89,1.11)	0.83(0.68,1.02)
CHDs	0.81(0.50,1.31)	0.72(0.44,1.17)	**0.69(0.50,0.94)**	**0.52(0.31,0.89)**
Limb defects	0.94(0.66,1.33)	0.75(0.53,1.07)	1.22(0.97,1.53)	0.90(0.61,1.33)
Orofacial clefts	0.69(0.32,1.48)	1.36(0.64,2.87)	1.48(0.86,2.53)	1.08(0.46,2.53)
Female fetus				
Birth defects	0.92(0.75,1.13)	1.06(0.86,1.30)	1.11(0.97,1.27)	0.86(0.68,1.09)
CHDs	1.06(0.62,1.82)	1.05(0.63,1.76)	**1.59(1.11,2.28)**	0.71(0.40,1.28)
Limb defects	0.86(0.54,1.38)	1.09(0.67,1.79)	1.01(0.75,1.37)	1.12(0.64,1.97)
Orofacial clefts	0.64(0.22,1.84)	0.84(0.28,2.54)	1.16(0.61,2.21)	2.99(0.77,11.70)
Autumn/Winter				
Birth defects	0.92(0.76,1.12)	1.07(0.89,1.28)	1.04(0.93,1.17)	0.98(0.79,1.22)
CHDs	0.94(0.57,1.55)	1.02(0.65,1.60)	1.22(0.91,1.63)	0.65(0.38,1.10)
Limb defects	0.97(0.65,1.45)	0.85(0.57,1.27)	1.06(0.84,1.35)	0.82(0.52,1.29)
Orofacial clefts	0.91(0.34,2.43)	1.54(0.55,4.28)	1.10(0.61,1.98)	**3.91(1.17,13.04)**
Summer/Spring				
Birth defects	**0.74(0.60,0.90)**	**0.80(0.65,0.97)**	1.02(0.89,1.17)	**0.73(0.58,0.92)**
CHDs	0.76(0.44,1.30)	0.76(0.44,1.32)	0.75(0.52,1.08)	0.61(0.33,1.13)
Limb defects	0.82(0.55,1.23)	0.83(0.55,1.26)	1.26(0.95,1.67)	1.12(0.71,1.77)
Orofacial clefts	0.56(0.24,1.30)	0.99(0.43,2.24)	1.62(0.89,2.95)	0.74(0.29,1.91)

Bold values represent statistical significance (two-sided $P<0.05$).

Abbreviations: CHDs, congenital heart defects; CI, confidence interval; NO_2, nitrogen dioxide; OR, odds ratio; PM_{10}, particulate matter ≤10 μm diameter; $PM_{2.5}$, particulate matter <2.5 μm diameter; SO_2, sulfur dioxide.

[a] Adjusted for year of conception, maternal age, gravidity, urban/rural, per capita area of roads, per capita of medication beds, unemployment, and per capita of cars.

Table S5 Adjusted odds ratios and 95% confidence interval of ambient air pollutants for the duration of peak level exposure during the 1st trimester and birth defects in Wuhan, China

Birth defects [a]	PM10 OR (95%CI)	PM2.5 OR (95%CI)	NO2 OR (95%CI)	SO2 OR (95%CI)
Total				
Birth defects	1.03(0.90,1.17)	1.10(0.94,1.28)	**1.40(1.26,1.57)**	1.04(0.86,1.27)
CHDs	0.91(0.67,1.23)	1.05(0.74,1.49)	**1.39(1.08,1.77)**	1.18(0.75,1.85)
Limb defects	1.02(0.74,1.41)	1.24(0.87,1.77)	**1.37(1.04,1.81)**	1.00(0.64,1.58)
Orofacial clefts	0.67(0.42,1.06)	0.92(0.54,1.54)	1.31(0.91,1.89)	0.90(0.47,1.73)
Male fetus				
Birth defects	1.08(0.90,1.29)	1.04(0.85,1.28)	**1.33(1.15,1.54)**	0.98(0.75,1.27)
CHDs	1.01(0.67,1.52)	1.03(0.64,1.65)	1.19(0.85,1.67)	1.11(0.59,2.11)
Limb defects	1.07(0.71,1.61)	1.18(0.75,1.85)	**1.48(1.05,2.08)**	0.85(0.47,1.52)
Orofacial clefts	0.67(0.37,1.22)	0.83(0.41,1.65)	1.17(0.73,1.87)	0.99(0.96,1.02)
Female fetus				
Birth defects	0.99(0.80,1.21)	1.17(0.93,1.47)	**1.52(1.29,1.80)**	1.08(0.81,1.44)
CHDs	0.78(0.50,1.23)	1.00(0.60,1.65)	**1.57(1.10,2.25)**	1.09(0.59,2.03)
Limb defects	0.96(0.56,1.63)	1.31(0.74,2.34)	1.21(0.76,1.95)	1.24(0.60,2.56)
Orofacial clefts	0.71(0.35,1.45)	1.01(0.46,2.23)	1.62(0.90,2.91)	1.01(0.39,2.61)
Autumn/Winter				
Birth defects	1.00(0.81,1.23)	1.24(0.99,1.55)	1.18(0.99,1.40)	1.12(0.78,1.61)
CHDs	1.25(0.75,2.08)	1.02(0.63,1.64)	1.46(0.97,2.19)	1.29(0.54,3.08)
Limb defects	1.01(0.59,1.70)	1.46(0.85,2.53)	1.22(0.77,1.94)	1.00(0.46,2.18)
Orofacial clefts	0.77(0.39,1.55)	0.99(0.48,2.03)	**2.31(1.15,4.66)**	1.48(0.50,4.41)
Summer/Spring				
Birth defects	1.03(0.78,1.36)	1.00(0.61,1.65)	1.23(1.00,1.52)	1.07(0.76,1.51)
CHDs	0.71(0.37,1.39)	0.66(0.16,2.83)	0.77(0.45,1.32)	1.52(0.69,3.37)
Limb defects	1.58(0.81,3.08)	2.17(0.92,5.08)	**1.61(1.01,2.57)**	0.92(0.42,2.02)
Orofacial clefts	0.35(0.12,1.04)	0.64(0.08,4.94)	0.59(0.25,1.39)	0.68(0.19,2.48)

Bold values represent statistical significance (two-sided P<0.05).

Abbreviations: CHDs, congenital heart defects; CI, confidence interval; NO2, nitrogen dioxide; OR, odds ratio; PM10, particulate matter ≤10 μm diameter; PM2.5, particulate matter <2.5 μm diameter; SO2, sulfur dioxide.

[a] Adjusted for year of conception, maternal age, gravidity, urban/rural, per capita area of roads, per capita of medication beds, unemployment, and per capita of cars.

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1-2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	5-6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	6
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*6*
Bias	9	Describe any efforts to address potential sources of bias	-
Study size	10	Explain how the study size was arrived at	-
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5-6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6
		(*b*) Describe any methods used to examine subgroups and interactions	6
		(*c*) Explain how missing data were addressed	-
		(*d*) If applicable, explain how loss to follow-up was addressed	-
		(*e*) Describe any sensitivity analyses	-
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6-7
		(b) Give reasons for non-participation at each stage	-
		(c) Consider use of a flow diagram	-
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	6-7
		(b) Indicate number of participants with missing data for each variable of interest	-
		(c) Summarise follow-up time (eg, average and total amount)	-
Outcome data	15*	Report numbers of outcome events or summary measures over time	6-7
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	-
		(*b*) Report category boundaries when continuous variables were categorized	8
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8-9
Discussion			
Key results	18	Summarise key results with reference to study objectives	10-11
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13
Generalisability	21	Discuss the generalisability (external validity) of the study results	12-13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Ambient air pollutants in the first trimester of pregnancy and birth defects: an observational study

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Ambient air pollutants in the first trimester of pregnancy and birth defects: an observational study

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Abstract

Objectives As current studies on the relationships between air pollutants exposure during the 1st trimester and birth defects were not fully elucidated, this study aimed to assess the association between selected air pollutants and birth defects.

Design An observational study.

Participants We obtained 70,854 singletons with gestational age <20 weeks who were delivered at a large maternal and child health care center in Wuhan, China.

Outcome measures Birth defects data and daily average concentration of ambient particulate matter ≤10 μm diameter (PM_{10}), particulate matter ≤2.5 μm diameter ($PM_{2.5}$), sulfur dioxide (SO_2), and nitrogen dioxide (NO_2) were obtained. Logistic regression analysis was applied to assess the association between maternal air pollutants exposure during 1st trimester and total birth defects, congenital heart defects (CHDs), limb defects, and orofacial clefts with adjustments of potential covariates.

Results There were a total of 1,352 birth defect cases included in this study, with a prevalence of 19.08‰. Maternal exposed to high concentrations of PM_{10}, $PM_{2.5}$, NO_2, and SO_2 in the 1st trimester were significantly associated with elevated odds ratios of birth defects (ORs ranged from 1.13 to 1.23). Additionally, for male fetuses, maternal exposed to high $PM_{2.5}$ concentration was associated with an elevated odd of CHDs (OR=1.27, 95% CI: 1.06, 1.52). In the cold season, the odds ratios of birth defects were significantly increased among women exposed to $PM_{2.5}$ (OR=1.64, 95% CI: 1.41, 1.91), NO_2 (OR=1.22, 95% CI: 1.08, 1.38), and SO_2 (OR=1.26, 95%CI: 1.07, 1.47).

Conclusions This study showed unfavorable effects of air pollutants exposure during the 1st trimester on birth defects. Especially, the association between maternal $PM_{2.5}$ exposure and CHDs was only observed among male fetuses, and stronger effects of $PM_{2.5}$, NO_2, and SO_2 exposure on birth defects were observed in the cold season.

Keywords: air pollutants; birth defects; congenital heart defects; limb defects; orofacial clefts; pregnancy

Strengths and limitations of this study

- This study explored the associations between maternal air pollutants exposure during 1st trimester and fetal outcomes classified by total birth defects, CHDs, limb defects, and orofacial clefts based on a large population.
- The stratification analysis by neonatal sex and season of conception indicated the specific high-risk population, which provided critical evidence of the air quality control policies.
- The selected participants are located in a large tertiary maternal care center in Wuhan and the representative of this study was undermined.
- Maternal air pollutants exposure indoor or in other living residents including work, dining, and shopping were not included, and other covariates including health behaviors and genetic factors were failed to obtain.

INTRODUCTION

Birth defects are structural or functional abnormalities occurred during embryonic development, most of them forming in the 1st trimester of pregnancy.[1] Birth defects are the leading cause of fetal death and are associated with the elevated risk of childhood mortality and reduced long-term survival.[2,3] According to the Institute for Health Metrics and Evaluation, the prevalence of congenital birth defects increased from 6.08% in 2005 to 6.29% in 2019.[4] Although the upward trends were reported in severe congenital heart defects, single ventricle, atrioventricular septal defects, and tetralogy of Fallot in Europe during 1980-2012, the prevalence of birth defects are much lower (1.0-4.1‰).[5] In China, the overall prevalence of birth defects increased from 12.83‰ in 1986 to 15.70‰ in 2014.[6] There is a necessity to explore the potential hazard factors contributed to the high prevalence of birth defects in China based on current knowledge.

Due to rapid urbanization, China has experienced severe air pollution in recent years. The Report on China's implementation of the Millennium Development Goals (2000-2015) documented that particulate matter ≤10 μm diameter (PM_{10}), sulfur dioxide (SO_2), and nitrogen dioxide (NO_2) were major air pollutants in urban areas.[7] Moreover, ambient particulate matter ≤2.5 μm diameter ($PM_{2.5}$) produced by coal combustion, industry sources, and vehicular emissions, is also one of the main air pollutants of industrialization.[8] The expanding coverage of ambient air pollutants surveillance has contributed to adverse pregnancy outcomes including small for gestational age, low birth weight, preterm birth, spontaneous abortion, and stillbirth in recent years.[9-11] Moreover, there is a growing interest in the associations between ambient air pollutants and birth defects, but the results of previous studies are controversial. Most studies reported that ambient air pollutants were associated with increased risks of birth defects.[12-14] However, Parkes et al. and Dolk et al. pointed out that high concentrations of PM_{10} and NO_2 exposure were not related to birth defects.[15,16] A recent meta-analysis conducted by Ma et al. even reported a protective effect of SO_2 on atrial septal defects.[17] These inconsistent results indicate that the effects of ambient air pollutants on birth defects could be varied by culture, ethnicity, or geographical distribution. More evidence is needed to clarify the risk of birth defects derived from air pollutants during the rapid social-economical development worldwide.

As current studies on the relationships between air pollutants and birth defects were not fully elucidated, this study aimed to assess the association between selected air pollutants and birth defects. Furthermore, the 1st trimester of pregnancy is vital for fetal development because fetal major organs and systems are formed at this stage and the fetus is most susceptible to environmental hazards. As a result, we mainly focused on the associations between maternal exposure to air pollutants including PM_{10}, $PM_{2.5}$, SO_2, and NO_2 during the 1st trimester and birth defects in Wuhan, China.

METHODS

Patient and public involvement

Patients or the public were not involved in any part of the design, conduct, reporting, or dissemination plans of this study.

Study design, site and population

This observational study was conducted in Wuhan city. Wuhan is the capital city of Hubei Province and a megacity in Central China. Its geographical location is 29° 58′ - 31° 22′ N and 113° 41′ - 115° 05′ E. The permanent resident population of Wuhan was over 10 million. Wuhan has four distinct seasons of hot summer and cold winter, with an annual average temperature of 15.8 ℃ - 17.5 ℃.[18]

There were a total of 130,186 perinatal women with detailed home addresses who delivered at the Maternal and Child Health Hospital of Hubei Province, and 98,877 of them lived in Wuhan City during the 1st trimester of pregnancy. Then we obtained 74,336 participants with distances less than 10 km from home to the nearest air surveillance station. After removing 3,333 mothers of multiple pregnancies and 149 with gestational age <20 weeks, 70,854 participants were finally included in this study.

Birth defects

Birth defect cases with gestational age ≥ 20 weeks and 0-7 days after birth including elective termination of pregnancy. Based on the requirements of the Maternal and

Child Health Monitoring Manual in China and the Implementation of National Hospital Birth Defects Surveillance of China, 23 types of common birth defects were categorized according to the 10th Revision of the International Classification of Diseases (ICD-10, Q00–Q99). The top three birth defects including congenital heart defects (CHDs) (Q20–Q28), limb defects (Q69–Q72), and orofacial clefts (Q35–Q37) were further examined by trained obstetricians, pediatricians, or pediatric cardiologists based on clinical observation, physical examination, biochemical index, and image examination results. Strict quality control of the reported data was performed by the assigned county-level inspector every quarter year and further checked by the city-level inspector semiannually. Detailed descriptions of the 23 types of birth defects were provided in Supplementary Table 1.

Exposure assessment

Data on the daily concentrations of air pollutants (PM_{10}, $PM_{2.5}$, NO_2, and SO_2) and the geographical locations (longitude and latitude) of the air surveillance stations were obtained from China's National Urban Air Quality Real-time Publishing Platform (http://106.37.208.233:20035). Moreover, the geographical location data was converted from the detailed home address of participants by Baidu Map. The distance from each home address of participants to all of the air surveillance stations were calculated. Then the individual daily air pollutants data were obtained according to the nearest surveillance station from home, and we only keep participants who lived within 10km from the nearest surveillance station, which was confirmed by other studies.[15,19] The median distance from home to the nearest station was 3.50 km in this study. Participants' average air pollutants exposure during the 1st trimester of pregnancy was estimated by mean levels of daily concentrations.

Statistical analysis

Observed outcomes were classified as total birth defects, CHDs, limb defects, and orofacial clefts. Maternal exposure variables including PM_{10}, $PM_{2.5}$, NO_2, and SO_2 were divided by interquartile range based on the daily average concentration during the 1st trimester. Covariates including the year of conception (2013-2018), maternal age (<25, 25-29, 30-34, and >34 years), gravidity (1, 2-3, and >3), and urban/rural were obtained. Moreover, other covariates including per capita of cars, unemployment, per capita area of roads, per capita of medication beds were retrieved from the official website of Wuhan Bureau of Statistics (http://tjj.wuhan.gov.cn/). Logistic regression analysis was applied to assess the association between maternal air pollutants exposure during 1st trimester and birth defects adjusted for potential covariates. Stratified analyses by neonatal sex (male and female fetus) and season of conception by last menstrual period (March to August and September to February) were applied to further explore the associations between air pollutants exposure in the 1st trimester of pregnancy and birth defects. The adjusted odds ratio (OR) and 95% confidence interval (CI) were provided in each model. Statistical analyses were performed by SAS 9.4 (SAS Institute, Inc., Cary, NC).

RESULTS

There were a total of 1,352 birth defect cases among 70,854 singletons, with a prevalence of 19.08‰ (Table 1). The prevalence of birth defects increased from 21.16‰ in 2013 to 24.08‰ in 2018. Subpopulations who conceived in winter (26.98‰), aged <25 years (28.10‰), or with gravidity >3 times (23.93‰) ranks the first place among corresponding categories. In addition, the male fetuses had a higher prevalence of birth defects than female fetuses (20.57‰ *vs* 16.80‰).

Table 1 Prevalence of birth defects among the subgroups of enrolled participants in Wuhan, China

Variables	Birth defect cases	Total cases	Prevalence (‰)
Year of conception			
2013	272	12,856	21.16
2014	203	11,236	18.07
2015	174	11,274	15.43
2016	227	12,261	18.51
2017	226	12,844	17.60
2018	250	10,383	24.08
Season of conception			
Spring	321	18,312	17.53
Summer	295	18,853	15.65
Autumn	287	17,045	16.84
Winter	449	16,644	26.98
Age (years)			

<25	122	4,341	28.10
25–29	632	34,412	18.37
30–34	401	23,783	16.86
>34	197	8,318	23.68
Gravidity			
1	604	35,590	16.97
2-3	372	19,552	19.03
>3	376	15,712	23.93
Neonatal sex			
Male	771	37,488	20.57
Female	560	33,337	16.80
Total	1,352	70,854	19.08

Table 2 shows the distributions of daily average ambient air pollutants concentrations in the 1^{st} trimester among the 5 groups including non-defects, birth defects, CHDs, limb defects, and orofacial clefts. The median exposures of PM_{10}, $PM_{2.5}$, NO_2, and SO_2 during the 1^{st} trimester were 101.78μg/m^3, 61.98μg/m^3, 53.64μg/m^3, and 13.97μg/m^3 respectively among the birth defects groups.

Table 2 Quartile concentrations (μg/m^3) of exposure for ambient air pollutants among birth defects groups in 1^{st} trimester of pregnancy in Wuhan, China

Air pollutants	Non-defects N=69,502	Birth defects N=1,352	CHDs N=265	Limb defects N=210	Orofacial clefts N=119
PM_{10}					
Min	36.86	39.08	49.82	40.76	48.85
25th	80.84	84.05	84.05	85.86	83.98
Median	102.42	101.78	99.46	105.80	96.77
75th	123.86	124.39	120.48	127.34	116.36
Max	231.49	225.91	225.91	220.82	191.37
$PM_{2.5}$					
Min	21.27	21.27	22.77	22.16	22.98
25th	39.37	45.02	44.88	42.63	41.26
Median	59.21	61.98	61.33	63.00	60.98
75th	80.20	80.73	78.81	84.66	77.38
Max	178.20	165.92	154.42	154.79	155.35
NO_2					
Min	9.71	15.22	29.03	25.78	16.89
25th	43.40	45.70	45.49	44.31	44.09
Median	51.49	53.64	53.69	53.43	53.84
75th	59.08	59.98	59.46	62.84	59.99
Max	93.60	93.37	92.93	93.37	87.58

SO_2					
Min	2.93	3.02	3.93	3.77	3.99
25th	9.07	9.36	9.43	10.55	9.46
Median	14.47	13.97	13.77	16.09	12.55
75th	23.54	23.87	23.36	25.23	21.28
Max	71.74	63.36	63.36	62.99	53.75

Abbreviations: CHDs, congenital heart defects; NO_2, nitrogen dioxide; PM_{10}, particulate matter ≤10 μm diameter; $PM_{2.5}$, particulate matter <2.5 μm diameter; SO_2, sulfur dioxide.

Table 3 presents the associations between ambient air pollutants exposure in the 1st trimester of pregnancy and birth defects. We also provided the odds ratios of birth defects in the 2nd and 3rd trimesters, as well as the entire pregnancy in the supplementary Tables S2-S4. High concentrate exposure to air pollutants was significantly associated with increased odds of total birth defects, yielding adjusted ORs of 1.13, 1.23, 1.18, and 1.19 for PM_{10}, $PM_{2.5}$, NO_2, and SO_2, respectively. Moreover, similar elevated odds of CHDs was observed for maternal exposure of $PM_{2.5}$ (OR=1.21, 95%CI: 1.06, 1.38), NO_2 (OR=1.13, 95%CI: 1.01, 1.27), and SO_2 (OR=1.24, 95%CI: 1.03, 1.48), but not PM_{10} (1.08, 95% CI: 0.95, 1.24).

For male fetuses, there were significantly increased odds of total birth defects among mothers exposed to PM_{10}, $PM_{2.5}$, NO_2, and SO_2 by interquartile increased concentrations during the 1st trimester (OR=1.13-1.23). Moreover, a positive association between $PM_{2.5}$ exposure during the 1st trimester and CHDs was detected (OR=1.27, 95% CI: 1.06, 1.52). For female fetuses, elevated odds of total birth defects (OR=1.14-1.22) were also observed among maternal exposed to heavy concentrated PM_{10}, $PM_{2.5}$, NO_2, and SO_2.

After stratified by season, the results show that the odds of total birth defects were significantly increased among women who conceived in Autumn/Winter and were exposed to $PM_{2.5}$ (OR=1.64, 95% CI: 1.41, 1.91), NO_2 (OR=1.22, 95% CI: 1.08, 1.38), or SO_2 (OR=1.26, 95% CI: 1.07, 1.47). $PM_{2.5}$, NO_2, and SO_2 were also positively related to CHDs (ORs=1.36-1.84). Elevated hazard of orofacial clefts was observed among women who conceived in the cold season exposed to heavy NO_2 concentrations (OR=1.85, 95% CI: 1.16, 2.93). However, in the warm season, the odd of total birth defects was elevated among women exposed to PM_{10} (OR=1.12, 95% CI: 1.00, 1.24).

Table 3 Adjusted odds ratios and 95% confidence interval of ambient air pollutants for each interquartile increase during the 1st trimester and birth defects in Wuhan, China

Birth defects [a]	PM_{10} OR (95%CI)	$PM_{2.5}$ OR (95%CI)	NO_2 OR (95%CI)	SO_2 OR (95%CI)
Total				
Birth defects	**1.13(1.07,1.21)**	**1.23(1.15,1.30)**	**1.18(1.12,1.24)**	**1.19(1.10,1.30)**
CHDs	1.08(0.95,1.24)	**1.21(1.06,1.38)**	**1.13(1.01,1.27)**	**1.24(1.03,1.48)**
Limb defects	1.11(0.96,1.29)	1.09(0.93,1.26)	**1.15(1.01,1.31)**	1.16(0.95,1.43)
Orofacial clefts	0.90(0.74,1.10)	1.04(0.85,1.26)	1.10(0.93,1.31)	0.95(0.73,1.22)
Male fetus				
Birth defects	**1.13(1.04,1.22)**	**1.23(1.14,1.34)**	**1.18(1.10,1.26)**	**1.19(1.07,1.33)**
CHDs	1.16(0.96,1.40)	**1.27(1.06,1.52)**	1.11(0.95,1.30)	1.18(0.93,1.50)
Limb defects	1.10(0.91,1.33)	1.08(0.90,1.30)	1.15(0.98,1.35)	1.14(0.89,1.47)
Orofacial clefts	0.86(0.66,1.12)	1.09(0.85,1.41)	1.07(0.86,1.34)	0.99(0.71,1.37)
Female fetus				
Birth defects	**1.14(1.04,1.25)**	**1.22(1.12,1.34)**	**1.19(1.10,1.29)**	**1.21(1.07,1.37)**
CHDs	0.99(0.82,1.20)	1.11(0.91,1.34)	1.14(0.97,1.34)	1.30(1.00,1.71)
Limb defects	1.08(0.84,1.38)	1.15(0.90,1.47)	1.15(0.93,1.43)	1.18(0.84,1.66)
Orofacial clefts	0.98(0.71,1.34)	0.95(0.69,1.30)	1.16(0.88,1.52)	0.88(0.59,1.30)
Autumn/Winter				
Birth defects	1.05(0.92,1.20)	**1.64(1.41,1.91)**	**1.22(1.08,1.38)**	**1.26(1.07,1.47)**
CHDs	1.05(0.79,1.39)	**1.84(1.32,2.57)**	**1.36(1.03,1.80)**	**1.43(1.00,2.03)**
Limb defects	1.19(0.84,1.70)	1.48(0.99,2.22)	1.35(0.97,1.87)	1.06(0.70,1.61)
Orofacial clefts	0.95(0.61,1.47)	1.63(0.98,2.70)	**1.85(1.16,2.93)**	1.40(0.82,2.40)
Summer/Spring				
Birth defects	**1.12(1.00,1.24)**	1.06(0.93,1.21)	1.09(1.00,1.19)	1.12(0.98,1.28)
CHDs	0.99(0.77,1.26)	0.89(0.65,1.21)	0.88(0.71,1.08)	1.24(0.92,1.66)
Limb defects	1.18(0.92,1.51)	1.21(0.89,1.63)	1.17(0.95,1.43)	1.23(0.89,1.71)
Orofacial clefts	0.72(0.50,1.03)	0.66(0.41,1.04)	0.88(0.65,1.19)	0.72(0.47,1.10)

Bold values represent statistical significance (two-sided $P<0.05$).

Abbreviations: CHDs, congenital heart defects; CI, confidence interval; NO_2, nitrogen dioxide; OR, odds ratio; PM_{10}, particulate matter ≤10 μm diameter; $PM_{2.5}$, particulate matter <2.5 μm diameter; SO_2, sulfur dioxide.

[a] Adjusted for year of conception, maternal age, gravidity, urban/rural, per capita area of roads, per capita of medication beds, unemployment, and per capita of cars.

DISCUSSION

In the current study, the prevalence of birth defects was close to what was reported in Hunan Province (19.18‰) and higher than that of Jiangsu Province (7.15‰).[20,21] Moreover, this study examined the associations between birth defects and air pollutants exposure in the 1st trimester, and we further explored the relationships stratified by fetal sex and season of conception.

This study showed that maternal exposure to ambient air pollutants including PM_{10}, $PM_{2.5}$, NO_2, and SO_2 during the 1st trimester could have higher odds of birth defects, which has been well documented previously. Moreover, we have adopted peak exposure on the thresholds at the 95th percentile to derive the accumulated days of high dose exposure, which further confirmed the positive association between high dose of NO_2 exposure during the 1st trimester and birth defects (Supplementary Table 5). A case-control study conducted by Al Noaimi et al. showed a positive association between $PM_{2.5}$ exposure in the 1st trimester of pregnancy and birth defects (OR=1.05).[22] Wang et al. applied Poisson generalized additive models on the time-series data adjusted for temperature, relative humidity, season, and time trend, which showed that maternal exposure to PM_{10} and NO_2 in early pregnancy significantly increased the risk of birth defects by 10.3% per 10 μg/m^3 and 3.4% per 10 μg/m^3, respectively.[23]

As was observed among the total participants, the risk of birth defects among both male fetuses and female fetuses was significantly related to PM_{10}, $PM_{2.5}$, NO_2, and SO_2. The underlying mechanisms between air pollutants exposure and the development of birth defects are still unclear. Maternal air pollutants exposures might cause changes in epigenetic signatures and permanent modifications in gene expression.[24] Animal studies showed that maternal exposure to $PM_{2.5}$ during pregnancy leads to spatial memory dysfunction, neurodevelopmental impairment, and disturbed cerebral cortex development of mice offspring.[25,26] Another study showed that maternal exposure to a high concentrate of $PM_{2.5}$ during the 1st trimester of pregnancy could result in the decreased placental expression of *BDNF* and *SYN1*, which may undermine fetal neurodevelopment.[27] Moreover, SO_2 is a systemic toxic agent which can induce chromosomal aberrations, sister chromatid exchanges, and micronuclei in mammalian cells.[28]

Except for PM_{10}, this study demonstrated adverse associations of CHDs with $PM_{2.5}$, NO_2, and SO_2 exposure. Previous studies in Taiwan and Northeast England also reported insignificant associations between PM_{10} exposure in the 1st trimester and CHDs,[29,30] whereas others confirmed the increased risk of CHDs related with PM_{10}.[12,23,31-34] Furthermore, Huang et al. showed that $PM_{2.5}$ exposure per each interquartile increase during gestational weeks 3-8 was related to an increased risk of CHDs (OR=1.21).[35] Additionally, the positive association between CHDs and maternal exposure to NO_2 and SO_2 was documented by studies conducted by Baldacci et al. and Vrijheid et al.[36,37] Moreover, a case-control study conducted by Jiang et al. showed that maternal exposure to SO_2 during the 1st trimester was significantly associated with increased risk of CHD (OR=1.78-2.04), and Hansen et al. also confirmed that a 0.6 ppb increase in SO_2 was associated with an increased risk of aortic artery and valve defects (OR=10.76).[19,38] The heterogeneity in these studies might be explained by the variation in the population, the gestational periods, or the measurement of air pollutants exposure. The physiopathological mechanism of the associations between air pollutants and CHDs was not fully elucidated. It is hypothesized that air pollutants exposure during the 1st pregnancy might strengthen the genetic and environmental interaction on CHDs.[37] Also, Air pollutants might change the molecule of DNA sequence or alter epigenetics related to CHDs.[39]

In line with this study, the epidemiological difference in CHD_S between male and female fetuses has been reported previously.[40,41] This disparity could be explained by the sex chromosome-linked genes expression and their interactions with hormonal effects during early development.[42] Interestingly, only male fetuses showed an elevated risk of CHDs who were exposed to a higher $PM_{2.5}$ concentration. This result indicates that $PM_{2.5}$ might emphasize the disparity in the embryonic origins of sexual dimorphism. Moreover, $PM_{2.5}$ might have a stronger effect on the expression of specific CHDs genes located on the Y chromosome. A study of mice models showed that increased pathological damage in the hearts of offspring mice was observed among maternal mice exposed to $PM_{2.5}$, and these effects in the male group were more obvious than in the female group. $PM_{2.5}$ exposure in utero inhibited the expression of the *GATA4* gene in male mice, which was related to the formation of CHDs.[43]

After stratified by season, maternal exposure of $PM_{2.5}$, NO_2, and SO_2 were positively associated with birth defects in the cold season instead of the warm season. Zhao et al. reported that the effects of air pollutants on birth defects were more obvious in the warm season in Hohhot.[44] This disparity could be partly explained by the uneven levels of dwellings' air pollutants. Compared to cities with lower GDP, cities with a higher GDP and a large population might have lower concentrations of indoor particulate matter.[45] Moreover, Wuhan is a well-known city with hot summer. Most of the residential buildings are equipped with air conditioners, which could help to improve indoor air quality in hot weather. Thus, the interpretation of results should be cautious that significant associations between air pollutants exposure and birth defects in the warm season in Wuhan.

Strengths

This study firstly provided evidence on the elevated risk of CHDs among mothers with heavy $PM_{2.5}$ exposure during the 1st trimester. We also found the increased risks of CHDs and orofacial clefts among women who conceived in the cold season and exposed to high concentrations of air pollutants.

Limitations

This study has several limitations. Firstly, the selected participants are located in a large tertiary maternal care center in Wuhan and the representative of this study was undermined. Secondly, the birth defects data of this study are manually collected and checked, but mistakes and omissions are inevitable. Thirdly, maternal air pollutants exposure indoor or in other living residents including work, dining, and shopping were not included in this study. Fourthly, other risk factors (including ethnicity, smoking, drinking, medications, drug use, and family history et al.) were failed to obtain, which might influence the interpretation of the relationship between maternal air pollutants exposure and birth defects. More research is needed to explore the pathogenic mechanism of air pollutants exposure during pregnancy and the associated birth defects.

CONCLUSIONS

Our study confirmed the unfavorable effects of maternal exposure to air pollutants (PM_{10}, $PM_{2.5}$, NO_2, and SO_2) on birth defects during the 1^{st} trimester of pregnancy. We firstly provided the evidence on the positive associations between $PM_{2.5}$ exposure and CHDs among male fetuses but not female fetuses. Moreover, stronger effects of $PM_{2.5}$, NO_2, and SO_2 exposure on birth defects were observed in the cold season in Wuhan. As a result, it should be noted for birth defects due to air pollutants, and reducing individual air pollutants exposure during the 1^{st} trimester might help to birth defect control in the context of the rapid development all over the world. Moreover, the implementation of air quality protection policies on birth defect control should consider seasonal factor, especially for the cold season in Wuhan, China. Future studies of birth defects and air quality data collected by individual air pollutants monitors are promoted.

Abbreviations

CHDs: congenital heart defects; CI: confidence interval; NO_2: nitrogen dioxide; OR: odds ratio; PM_{10}: particulate matter ≤10 μm diameter; $PM_{2.5}$: particulate matter ≤2.5 μm diameter; SO_2: sulfur dioxide.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Maternal and Child Health Hospital of Hubei Province (2021IECLW025). This study was based on the retrospective clinical data without any individual patient identifiers.

Patient consent for publication

Not applicable.

Patient and public involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

Availability of data and materials

The data used in this study are available from corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Yao Cheng and Jieyun Yin contributed equally to this manuscript. Yao Cheng collected the data, performed data analysis, draft and revised the manuscript. Jieyun Yin collected the data, performed data analysis, and edit the manuscript. Lijun Yang designed the study, collected the data, and performed data analysis. Man Xu, Xinfeng Lu, and Wenting Huang collected the data, interpreted the data, and reviewed the manuscript. Guohong Dai: collected the data, interpreted the data, and revised the manuscript. Guoqiang Sun: designed the study, collected the data, and supervised the project. All authors gave final approval of the version to be submitted and agreed to be accountable for all aspects of the work.

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Table S1 The rank result of the 23 types of common birth defects

Number	Birth defect	N
21	Conjoined twins	0
13	Bladder valgus	1
1	Anencephaly	2
3	Encephalocele	3
20	Gastroschisis	3
8	Small ears (including no ears)	6
10	Esophageal atresia or stenosis	8
2	Spina bifida	12
18	Congenital diaphragmatic hernia	13
19	Omphalocele	18
12	Hypospadias	27
11	Rectoanal atresia or stricture (including anorectal atresia)	30
14	Equinovarus	41
4	Congenital hydrocephalus	45
22	Down's syndrome	79
9	Other malformations of the external ear (except for small ears and no ears)	86
5, 6, 7	Orofacial clefts	119
15, 16, 17	Limb defects	210
23	Congenital heart defects	265

Table S2 Adjusted odds ratios and 95% confidence interval of ambient air pollutants for each interquartile increase during the 2[ed] trimester and birth defects in Wuhan, China

Birth defects [a]	PM_{10} OR (95%CI)	$PM_{2.5}$ OR (95%CI)	NO_2 OR (95%CI)	SO_2 OR (95%CI)
Total				
Birth defects	1.01(0.93,1.09)	0.95(0.89,1.02)	0.99(0.93,1.06)	**0.88(0.80,0.97)**
CHDs	0.98(0.79,1.22)	0.86(0.72,1.04)	0.94(0.79,1.11)	**0.75(0.58,0.97)**
Limb defects	1.03(0.87,1.22)	0.95(0.82,1.11)	1.05(0.92,1.21)	1.01(0.83,1.25)
Orofacial clefts	1.27(0.86,1.87)	**1.42(1.01,1.99)**	1.27(0.93,1.74)	1.38(0.85,2.25)
Male fetus				
Birth defects	1.01(0.91,1.12)	0.95(0.87,1.04)	0.95(0.87,1.03)	0.90(0.79,1.02)
CHDs	0.82(0.61,1.10)	**0.69(0.52,0.91)**	**0.69(0.53,0.88)**	**0.58(0.41,0.83)**
Limb defects	1.12(0.90,1.38)	1.00(0.84,1.20)	1.09(0.92,1.29)	1.09(0.84,1.41)
Orofacial clefts	1.11(0.69,1.81)	1.20(0.79,1.82)	1.25(0.83,1.87)	1.19(0.67,2.12)
Female fetus				
Birth defects	1.01(0.89,1.14)	0.96(0.86,1.07)	1.06(0.96,1.17)	0.87(0.75,1.00)
CHDs	1.21(0.88,1.66)	1.07(0.82,1.39)	1.28(0.99,1.65)	1.00(0.69,1.44)
Limb defects	0.90(0.69,1.18)	0.87(0.68,1.12)	0.99(0.79,1.25)	0.89(0.63,1.25)
Orofacial clefts	1.64(0.80,3.36)	**2.02(1.06,3.86)**	1.31(0.79,2.18)	2.01(0.75,5.39)
Summer/Spring				
Birth defects	1.04(0.93,1.17)	0.91(0.82,1.01)	1.03(0.94,1.13)	0.92(0.79,1.06)
CHDs	0.99(0.73,1.33)	0.79(0.60,1.04)	1.01(0.78,1.29)	**0.64(0.44,0.94)**
Limb defects	1.10(0.86,1.41)	0.88(0.69,1.11)	1.06(0.87,1.29)	0.92(0.67,1.25)
Orofacial clefts	1.20(0.64,2.25)	1.47(0.81,2.67)	1.07(0.66,1.76)	1.71(0.74,3.97)
Autumn/Winter				
Birth defects	0.96(0.86,1.08)	1.00(0.91,1.10)	0.97(0.89,1.07)	0.87(0.75,1.00)
CHDs	1.02(0.74,1.40)	1.00(0.76,1.30)	0.93(0.72,1.19)	1.00(0.68,1.47)
Limb defects	0.94(0.74,1.18)	0.99(0.82,1.21)	1.05(0.87,1.28)	1.05(0.77,1.43)
Orofacial clefts	1.30(0.78,2.17)	1.34(0.88,2.03)	1.38(0.90,2.12)	1.08(0.58,2.03)

Bold values represent statistical significance (two-sided $P<0.05$).

Abbreviations: CHDs, congenital heart defects; CI, confidence interval; NO_2, nitrogen dioxide; OR, odds ratio; PM_{10}, particulate matter ≤10 μm diameter; $PM_{2.5}$, particulate matter <2.5 μm diameter; SO_2, sulfur dioxide.

[a] Adjusted for year of conception, maternal age, gravidity, urban/rural, per capita area of roads, per capita of medication beds, unemployment, and per capita of cars.

Table S3 Adjusted odds ratios and 95% confidence interval of ambient air pollutants for each interquartile increase during the 3rd trimester and birth defects in Wuhan, China

Birth defects [a]	PM_{10} OR (95%CI)	$PM_{2.5}$ OR (95%CI)	NO_2 OR (95%CI)	SO_2 OR (95%CI)
Total				
Birth defects	**0.86(0.81,0.92)**	**0.89(0.84,0.94)**	**0.90(0.85,0.95)**	**0.92(0.86,0.98)**
CHDs	0.90(0.79,1.03)	**0.87(0.77,0.98)**	**0.89(0.79,1.00)**	0.88(0.76,1.01)
Limb defects	0.96(0.83,1.12)	0.90(0.79,1.04)	0.97(0.85,1.10)	1.00(0.85,1.17)
Orofacial clefts	**0.78(0.64,0.95)**	0.97(0.81,1.16)	0.90(0.76,1.07)	0.89(0.72,1.10)
Male fetus				
Birth defects	**0.87(0.80,0.94)**	**0.88(0.81,0.94)**	**0.91(0.85,0.97)**	0.96(0.88,1.05)
CHDs	0.85(0.72,1.01)	0.88(0.74,1.05)	0.88(0.76,1.03)	**0.81(0.67,0.98)**
Limb defects	0.93(0.77,1.11)	0.89(0.75,1.05)	0.92(0.79,1.08)	1.03(0.85,1.25)
Orofacial clefts	**0.78(0.60,1.00)**	0.94(0.75,1.19)	0.93(0.75,1.16)	0.95(0.72,1.25)
Female fetus				
Birth defects	**0.86(0.78,0.94)**	**0.91(0.84,0.99)**	**0.89(0.83,0.96)**	**0.86(0.78,0.95)**
CHDs	0.93(0.77,1.14)	0.92(0.76,1.10)	0.91(0.77,1.07)	0.98(0.79,1.21)
Limb defects	1.01(0.79,1.30)	0.94(0.74,1.19)	1.05(0.85,1.31)	0.93(0.72,1.21)
Orofacial clefts	0.78(0.57,1.08)	0.99(0.74,1.32)	0.85(0.66,1.10)	0.79(0.56,1.11)
Summer/Spring				
Birth defects	1.04(0.94,1.16)	**1.18(1.07,1.31)**	1.06(0.98,1.16)	0.96(0.87,1.06)
CHDs	1.07(0.84,1.36)	1.16(0.91,1.46)	1.13(0.94,1.37)	0.84(0.68,1.05)
Limb defects	1.06(0.82,1.37)	0.89(0.66,1.19)	0.97(0.78,1.21)	0.94(0.74,1.19)
Orofacial clefts	0.96(0.66,1.39)	1.37(0.98,1.91)	1.22(0.91,1.64)	0.93(0.67,1.30)
Autumn/Winter				
Birth defects	**0.64(0.56,0.73)**	**0.78(0.70,0.87)**	**0.74(0.66,0.82)**	0.86(0.73,1.00)
CHDs	**0.74(0.55,0.99)**	**0.73(0.57,0.94)**	**0.65(0.52,0.82)**	0.82(0.58,1.15)
Limb defects	0.78(0.57,1.08)	0.82(0.62,1.08)	1.05(0.78,1.41)	1.07(0.73,1.58)
Orofacial clefts	0.78(0.55,1.10)	**0.40(0.27,0.60)**	**0.52(0.38,0.70)**	**0.62(0.39,0.99)**

Bold values represent statistical significance (two-sided $P<0.05$).

Abbreviations: CHDs, congenital heart defects; CI, confidence interval; NO_2, nitrogen dioxide; OR, odds ratio; PM_{10}, particulate matter ≤10 μm diameter; $PM_{2.5}$, particulate matter <2.5 μm diameter; SO_2, sulfur dioxide.

[a] Adjusted for year of conception, maternal age, gravidity, urban/rural, per capita area of roads, per capita of medication beds, unemployment, and per capita of cars.

Table S4 Adjusted odds ratios and 95% confidence interval of ambient air pollutants for each interquartile increase during the entire pregnancy and birth defects in Wuhan, China

Birth defects [a]	PM_{10} OR (95%CI)	$PM_{2.5}$ OR (95%CI)	NO_2 OR (95%CI)	SO_2 OR (95%CI)
Total				
Birth defects	**0.85(0.74,0.97)**	0.95(0.83,1.09)	1.04(0.95,1.13)	**0.84(0.72,0.98)**
CHDs	0.91(0.64,1.29)	0.86(0.60,1.23)	1.01(0.80,1.27)	**0.60(0.41,0.89)**
Limb defects	0.91(0.69,1.21)	0.85(0.64,1.13)	1.14(0.95,1.37)	0.97(0.71,1.34)
Orofacial clefts	0.68(0.36,1.26)	1.16(0.62,2.17)	1.34(0.89,2.04)	1.45(0.70,2.98)
Male fetus				
Birth defects	**0.81(0.68,0.97)**	0.89(0.75,1.06)	0.99(0.89,1.11)	0.83(0.68,1.02)
CHDs	0.81(0.50,1.31)	0.72(0.44,1.17)	**0.69(0.50,0.94)**	**0.52(0.31,0.89)**
Limb defects	0.94(0.66,1.33)	0.75(0.53,1.07)	1.22(0.97,1.53)	0.90(0.61,1.33)
Orofacial clefts	0.69(0.32,1.48)	1.36(0.64,2.87)	1.48(0.86,2.53)	1.08(0.46,2.53)
Female fetus				
Birth defects	0.92(0.75,1.13)	1.06(0.86,1.30)	1.11(0.97,1.27)	0.86(0.68,1.09)
CHDs	1.06(0.62,1.82)	1.05(0.63,1.76)	**1.59(1.11,2.28)**	0.71(0.40,1.28)
Limb defects	0.86(0.54,1.38)	1.09(0.67,1.79)	1.01(0.75,1.37)	1.12(0.64,1.97)
Orofacial clefts	0.64(0.22,1.84)	0.84(0.28,2.54)	1.16(0.61,2.21)	2.99(0.77,11.70)
Autumn/Winter				
Birth defects	0.92(0.76,1.12)	1.07(0.89,1.28)	1.04(0.93,1.17)	0.98(0.79,1.22)
CHDs	0.94(0.57,1.55)	1.02(0.65,1.60)	1.22(0.91,1.63)	0.65(0.38,1.10)
Limb defects	0.97(0.65,1.45)	0.85(0.57,1.27)	1.06(0.84,1.35)	0.82(0.52,1.29)
Orofacial clefts	0.91(0.34,2.43)	1.54(0.55,4.28)	1.10(0.61,1.98)	**3.91(1.17,13.04)**
Summer/Spring				
Birth defects	**0.74(0.60,0.90)**	**0.80(0.65,0.97)**	1.02(0.89,1.17)	**0.73(0.58,0.92)**
CHDs	0.76(0.44,1.30)	0.76(0.44,1.32)	0.75(0.52,1.08)	0.61(0.33,1.13)
Limb defects	0.82(0.55,1.23)	0.83(0.55,1.26)	1.26(0.95,1.67)	1.12(0.71,1.77)
Orofacial clefts	0.56(0.24,1.30)	0.99(0.43,2.24)	1.62(0.89,2.95)	0.74(0.29,1.91)

Bold values represent statistical significance (two-sided P<0.05).

Abbreviations: CHDs, congenital heart defects; CI, confidence interval; NO_2, nitrogen dioxide; OR, odds ratio; PM_{10}, particulate matter ≤10 μm diameter; $PM_{2.5}$, particulate matter <2.5 μm diameter; SO_2, sulfur dioxide.

[a] Adjusted for year of conception, maternal age, gravidity, urban/rural, per capita area of roads, per capita of medication beds, unemployment, and per capita of cars.

Table S5 Adjusted odds ratios and 95% confidence interval of ambient air pollutants for the duration of peak level exposure during the 1st trimester and birth defects in Wuhan, China

Birth defects [a]	PM10 OR (95%CI)	PM2.5 OR (95%CI)	NO2 OR (95%CI)	SO2 OR (95%CI)
Total				
Birth defects	1.03(0.90,1.17)	1.10(0.94,1.28)	**1.40(1.26,1.57)**	1.04(0.86,1.27)
CHDs	0.91(0.67,1.23)	1.05(0.74,1.49)	**1.39(1.08,1.77)**	1.18(0.75,1.85)
Limb defects	1.02(0.74,1.41)	1.24(0.87,1.77)	**1.37(1.04,1.81)**	1.00(0.64,1.58)
Orofacial clefts	0.67(0.42,1.06)	0.92(0.54,1.54)	1.31(0.91,1.89)	0.90(0.47,1.73)
Male fetus				
Birth defects	1.08(0.90,1.29)	1.04(0.85,1.28)	**1.33(1.15,1.54)**	0.98(0.75,1.27)
CHDs	1.01(0.67,1.52)	1.03(0.64,1.65)	1.19(0.85,1.67)	1.11(0.59,2.11)
Limb defects	1.07(0.71,1.61)	1.18(0.75,1.85)	**1.48(1.05,2.08)**	0.85(0.47,1.52)
Orofacial clefts	0.67(0.37,1.22)	0.83(0.41,1.65)	1.17(0.73,1.87)	0.99(0.96,1.02)
Female fetus				
Birth defects	0.99(0.80,1.21)	1.17(0.93,1.47)	**1.52(1.29,1.80)**	1.08(0.81,1.44)
CHDs	0.78(0.50,1.23)	1.00(0.60,1.65)	**1.57(1.10,2.25)**	1.09(0.59,2.03)
Limb defects	0.96(0.56,1.63)	1.31(0.74,2.34)	1.21(0.76,1.95)	1.24(0.60,2.56)
Orofacial clefts	0.71(0.35,1.45)	1.01(0.46,2.23)	1.62(0.90,2.91)	1.01(0.39,2.61)
Autumn/Winter				
Birth defects	1.00(0.81,1.23)	1.24(0.99,1.55)	1.18(0.99,1.40)	1.12(0.78,1.61)
CHDs	1.25(0.75,2.08)	1.02(0.63,1.64)	1.46(0.97,2.19)	1.29(0.54,3.08)
Limb defects	1.01(0.59,1.70)	1.46(0.85,2.53)	1.22(0.77,1.94)	1.00(0.46,2.18)
Orofacial clefts	0.77(0.39,1.55)	0.99(0.48,2.03)	**2.31(1.15,4.66)**	1.48(0.50,4.41)
Summer/Spring				
Birth defects	1.03(0.78,1.36)	1.00(0.61,1.65)	1.23(1.00,1.52)	1.07(0.76,1.51)
CHDs	0.71(0.37,1.39)	0.66(0.16,2.83)	0.77(0.45,1.32)	1.52(0.69,3.37)
Limb defects	1.58(0.81,3.08)	2.17(0.92,5.08)	**1.61(1.01,2.57)**	0.92(0.42,2.02)
Orofacial clefts	0.35(0.12,1.04)	0.64(0.08,4.94)	0.59(0.25,1.39)	0.68(0.19,2.48)

Bold values represent statistical significance (two-sided $P<0.05$).

Abbreviations: CHDs, congenital heart defects; CI, confidence interval; NO2, nitrogen dioxide; OR, odds ratio; PM10, particulate matter ≤10 μm diameter; PM2.5, particulate matter <2.5 μm diameter; SO2, sulfur dioxide.

[a] Adjusted for year of conception, maternal age, gravidity, urban/rural, per capita area of roads, per capita of medication beds, unemployment, and per capita of cars.

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1-2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	5-6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	6
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*6*
Bias	9	Describe any efforts to address potential sources of bias	-
Study size	10	Explain how the study size was arrived at	-
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5-6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6
		(*b*) Describe any methods used to examine subgroups and interactions	6
		(*c*) Explain how missing data were addressed	-
		(*d*) If applicable, explain how loss to follow-up was addressed	-
		(*e*) Describe any sensitivity analyses	-
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6-7
		(b) Give reasons for non-participation at each stage	-
		(c) Consider use of a flow diagram	-
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	6-7
		(b) Indicate number of participants with missing data for each variable of interest	-
		(c) Summarise follow-up time (eg, average and total amount)	-
Outcome data	15*	Report numbers of outcome events or summary measures over time	6-7
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	-
		(*b*) Report category boundaries when continuous variables were categorized	8
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8-9
Discussion			
Key results	18	Summarise key results with reference to study objectives	10-11
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13
Generalisability	21	Discuss the generalisability (external validity) of the study results	12-13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.