MATERNAL-PERINATAL RISK AND CHILDREN-ADOLESCENT HEALTH

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MATERNAL-PERINATAL RISK AND CHILDREN-ADOLESCENT HEALTH

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EDITORIAL
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Editorial: Maternal-Perinatal Risk and Children-Adolescent Health

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Keywords: maternal-perinatal risk, children health, adolescent health, social stress, inflammatory response

Editorial on the Research Topic

Maternal-Perinatal Risk and Children-Adolescent Health

Environmental and behavioral factors during pregnancy and perinatal periods have been associated with the future development of health disorders, particularly with non-communicable chronic diseases. Maternal infections, nutrient deficiencies, and social stresses have been described as factors that are highly relevant in low and medium-income countries (1). The present issue of Frontiers has been dedicated to explore the topic Maternal-Perinatal Risk and Children-Adolescent Health, which gave us the opportunity to examine this topic with different approaches. In addition, the results of the articles published in the present issue open doors for future research and interventions in this important topic.

The paper of Chang et al. analyzed a big database from Taiwan, one of the leading countries with outstanding assistance in reproductive technology and modern medical care, and reported a higher rate of low birth and preterm birth as compared to those in the natural pregnancy group. This suggested that further evaluation and tighter regulations may be needed with regards to the use of this technology. In this context, Mei et al. described that in Traditional Chinese Medicine, the herbal recipe *Bushen Yutai* was able to increase and produce higher quality embryos, both of which have important implications to *in vitro* reproduction. The results of González-Fernández et al. showed that maternal stress in women from a conflict zone was associated with biomarkers of maternal-fetal well-being, highlighting the importance of assessing and addressing stress in pregnant women from vulnerable communities. Torres-Muñoz et al. described, in low-income pregnant women, that the assessment of biological and psychosocial factors and risk behaviors was important to determine the risk of developing perinatal asphyxia. Moreover, the same group showed that late premature infants were products of mothers with morbidity (specifically hypertensive disorders), had greater odds to be born by cesarean section, had a higher probability of developing morbidity, and more often, needed early obstetric intervention and required further resuscitation. Moreover, Torres-Salazar et al. found in women with preeclampsia differential methylation in the glucocorticoid receptor (GR) NR3C1 and its co-chaperone HSP90AA1. They suggested a possible regulatory role of this receptor in the response to stress during pregnancy and also a physio-pathological role in preeclampsia. In order to investigate factors impacting the risk of child maltreatment, Kawaguchi et al. revealed that child maltreatment was associated with maternal younger age, fathers much older than mothers, and single mothers. The evidence suggested a causal relationship between non-sexual child maltreatment and a range of mental disorders, drug use, suicide attempts, sexually-transmitted infections, and risky sexual behavior during childhood and into adulthood. All forms of child maltreatment should be considered as important risk factors to

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illness with a sizeable impact on major contributors to the burden of disease in all countries (2).

Obesity and overweight are linked with gestational diabetes and preeclampsia, the most important causes of maternal and perinatal morbidity and mortality in developing countries. Liu et al. presented the results of a meta-analysis demonstrating that pre-pregnancy maternal overweight or obesity and also high or low gestational weight gain rendered their offspring susceptible to an increased risk of asthma and wheeze during childhood. These results demonstrated the importance of appropriate antenatal health that includes maternal nutritional evaluation. The importance of maternal nutrition and child health is highlighted by the results of Yu et al., who reported that vitamin D supplementation during pregnancy could effectively reduce the risk of its deficiency in neonates. Adequate nutrition during pregnancy is important for fetal growth and for better maternal immunity response. Martinez-Lopez et al. demonstrated that the eating behavior of a family is determined by the meaning that the caretaker gives to food, by the act of eating in the domestic environment, and by beliefs and perceptions around those concepts. The common domains are well-being, health maintenance, coexistence, and security. Pregnancy has adverse effects on the prognosis of cervical cancer. Li et al. described the epidemiological characteristics, clinical features, clinical management, and maternal and infant outcomes of patients with cervical cancer during pregnancy. They found that an early diagnosis improved the prognosis of cervical cancer during pregnancy.

In a second article from Gonzalez-Fernandez et al., the association of maternal infections, nutrient deficiencies, and inflammation (MINDI) with maternal blood pressure and symphysis-fundal-height were explored, showing that mean arterial blood pressure and pulse pressure were useful tools to detect women at risk of adverse pregnancy outcomes in settings that have limited access to technology. In this context, Samwel et al. reported that bacterial vaginosis (BV) predisposed women and their babies to an increased frequency of illness. They showed a higher frequency of gastrointestinal morbidity among BV-HIV-1-exposed infants, observing that at 6 and 12 months, infants from mothers with BV had higher odds of bloody stool dehydration, vomiting, and mouth ulcers.

The impact of early life stress on postnatal brain development has been reviewed by González-Acosta et al. The understanding of this relationship has allowed for a better formulation and implementation of preventive measures, as well as the reorientation of research targets to improve health outcomes in all populations. Wang et al. observed a significant geographic disparity and an association between anemia and stunting among specific groups of school-aged children in China and thereby suggested that eliminating the geographic disparity and ameliorating stunting might contribute to the improvement of anemia, especially in adolescent girls and in groups with serious anemia burden. In a prospective birth cohort study in Shanghai, Li et al. described the impact of early life pet ownership on allergic sensitization and atopic dermatitis showing that half of the preschool children had positive allergen sensitization even in non-atopic children. Although early life exposure to dogs may increase the risk of allergic sensitization, it significantly decreased the risk of atopic dermatitis.

Early life stress can be caused by acute or chronic exposure to childhood events, such as emotional, physical, sexual abuse, and neglect. Early life stress is associated with subsequent alterations in physical and mental health, which can extend into adolescence, adulthood, and older age. The evidence suggests a causal relationship between non-sexual child maltreatment and a range of mental disorders, drug use, suicide attempts, sexually-transmitted infections, and risky sexual behavior during childhood and adulthood (2, 3) as discussed by Gonzalez et al. in this special issue.

Biomedical and psychosocial risk factors during pregnancy increase inflammatory response involved in the development of maternal diseases affecting fetal growth (3). Low birth weight is a marker of poor fetal growth and nutrition, and it is linked to coronary artery disease, hypertension, obesity, and insulin resistance (4). Early assessment and intervention of biomedical and psychosocial risk can prevent perinatal mortality, morbidity, and prevent child maltreatment (5). This special issue reported new potential links between maternal-perinatal risk environmental factors and children and adolescent health. The heterogeneity of articles published in this special topic highlights that maternal, perinatal, and children and adolescent health need to be approached in a multidisciplinary fashion.

AUTHOR CONTRIBUTIONS

JH-M, PL-J, KK, and AS were the associate editors. JH-E was editorial author. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Lopez-Jaramillo P, Gomez-Arbelaez D, Sotomayor-Rubio A, Mantilla-Garcia D, Lopez-Lopez J. Maternal undernutrition and cardiometabolic disease: A Latin America Perspective. *BMC Med.* (2015) 13:41. doi: 10.1186/s12916-015-0293-8
2. Norman RE, Byambaa M, De Rumna, Butchart A, Scott J, Vos T. The long-term health consequences of child physical abuse, emotional abuse, and neglect: a systematic review and meta-analysis. *PLoS Med.* (2012) 9:e1001349. doi: 10.1371/journal.pmed.1001349
3. Salmeron B, Herrera JA, Hurtado H. The biopsychosocial risk assessment and the low birthweight. *Soc Sci Med.* (1997) 44:1107–14. doi: 10.1016/S0277-9536(96)00240-7
4. Calkins K, Devaskar SU. Fetal origins of adult disease. *Curr Probl Curr Probl Pediatr Adolesc Health Care.* (2011) 41:158–76. doi: 10.1016/j.cppeds.2011.01.001

5. Choi KW, Sikkema KJ. Childhood maltreatment and perinatal mood and anxiety disorders: a systematic review. *Trauma Violence Abuse.* (2016) 17:427–53. doi: 10.1177/1524838015584369

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BRIEF RESEARCH REPORT
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Children Conceived by Assisted Reproductive Technology Prone to Low Birth Weight, Preterm Birth, and Birth Defects: A Cohort Review of More Than 50,000 Live Births During 2011–2017 in Taiwan

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Objectives: The use of assisted reproductive technology (ART) has increased rapidly in Taiwan. The purpose of this study is to discuss the risks of low birth weight, preterm birth, and birth defect for children conceived by assisted reproductive technology in Taiwan.

Methods: Both National ART report database and National birth reports were obtained from the Health Promotion Administration in the Ministry of Health and Welfare in Taiwan. The cohort included live births (n = 1,405,625) and children conceived by ART (n = 50,988/172,818 cycles) from 2011 to 2017. The prevalence of low birth weight, preterm birth, and birth defect were compared between the ART and natural pregnancy groups.

Results: Children conceived by ART displayed a higher rate of low birth weight as compared to those in the natural pregnancy group ($p < 0.001$), even when analyses were restricted to singleton births ($p < 0.001$). A higher rate of preterm birth ($p < 0.001$) was also observed in children conceived by ART even when analyses were restricted to singleton births ($p < 0.05$). A significant increased rate of birth defects was noted from children conceived by ART ($p < 0.05$).

Conclusions: With the increasing need for and use of ART-conceptions, the likelihood of risks induced or related to Assistant Reproductive Technology (ART) has drawn considerable attention in recent years. Taiwan, as one of the leading countries with outstanding ART performances and modern medical care, the result of the current study suggests that further consideration and tighter regulations and policy are needed with regard to the use of ART.

Keywords: assisted reproductive technology, lower birth weight, preterm birth, birth defects, live births

INTRODUCTION

The first *in vitro* fertilization baby in Taiwan was born in 1985, and thereafter, the number of Assisted Reproductive Technology (ART) cycles has increased rapidly. The percentage of births by ART increased from 0.86% in 1998 to 1.44% in 2007, and then to 4.33% in 2016, with an annual increase of 41.23% (1). Possible social factors contributing to the increase of using ART are many; such as women postponing their childbearing age, busy jobs, higher social pressure, or even same-sex marriages. The government policy also supports the use of ART in the face of the decreasing birth rate in Taiwan. However, health risks associated with ART-conceptions have been reported in both animal studies and epidemiological surveys, which demands more attention and further considerations in future clinic practices.

Studies have revealed that ART increased the risks of preterm birth, low birth weight, congenital anomalies, and perinatal mortality as compared to that of natural pregnancies (2–6). The occurrences of preterm birth and low birth weight were still high in singleton births by ART (7). An increased incidence of preterm birth has been reported (8), and the risk could not be reduced even by elective single embryo transfer (9). Hansen et al. performed a meta-analysis of publications before 2003, and concluded that birth defects increased in children conceived by ART (10).

Sandin et al. studied more than 2.5 million children born in Sweden from 1982 through 2007 and found that the risk of mental retardation was significantly higher in children conceived by ART as compared to their counterparts through natural pregnancies (11). Fountain et al. studied 6 million live births and found that the incidence of autism was 2-fold as higher as in children conceived by ART than in natural pregnancy (12). Hansen et al. studied more than 210,000 children born in Western Australia between 1994 and 2002 and found an increased risk of intellectual disability for children conceived by ART (10). Researchers also reported an increase in arterial hypertension in children conceived by ART, probably due to ART-induced premature vascular aging (13, 14). Fetus conceived by ART also showed signs of cardiovascular remodeling, including a more globular heart, thick myocardial walls, decreased longitudinal functions, impaired relaxation, and dilated atria (15). An increased risks of cancer has also been observed in children conceived by ART (16–18).

In spite of the risks observed in children conceived by ART, the use of ART has increased rapidly in Taiwan. The aim of this study is to investigate the outcomes of children conceived by ART during 2011–2017 in Taiwan.

METHODS

Data Sources

All the data of mothers and babies was obtained from a website-based reporting system designed by the Ministry of Health and Welfare in Taiwan and approved by the Ethics Committee of Taipei Hospital, Ministry of Health and Welfare (TH-IRB-0020-0002). Since 2007, the Ministry of Health and Welfare, MOHW (previously known as the Department of Health under the Executive Yuan) has requested all participating ART clinics and hospitals to register cycle-specific information for all ART treatment cycles. Such information includes patient characteristics, information on ART treatments, the pregnancy rate and obstetric outcomes. Both the National ART report database and National birth reports, which includes the yearly number of people by age and gender and national annual number of births, were obtained from the Health Promotion Administration in the Ministry of Health and Welfare. We therefore took advantage of this system to collaborate with MOHW, our cohort included natural pregnancy live births ($n = 1{,}405{,}625$) and children conceived by ART ($n = 50{,}988/172{,}818$ cycles) from 2011 to 2017.

Measures and Statistical Analysis

We obtained and calculated the following variables: (1) rate of very low birth weight (VLBW, < 1,500 g); number of VLBW by ART divided by national number of ART in the same year, (2) rate of low birth weight (LBW, 1,500–2,499 g); number of LBW by ART divided by national number of ART in the same year, (3) rate of preterm birth (gestational age < 37 weeks); number of preterm births by ART divided by national number of ART in the same year, and (4) rate of birth defects (cardiovascular, central nervous system, gastrointestinal, urogenital, musculoskeletal, ear and facial and chromosome defects) by ART; number of birth defects by ART divided by national number of ART in the same year. We also obtained and calculated the same variables for the natural pregnancies and compared with those of ART conceptions. We applied a time series design for the measurements by *T*-Test.

RESULTS

Demographic Characteristics of the Cohorts

The percentage of births by ART increased from 2.77% in 2011 to 4.92% in 2017 (**Supplement Figure 1**). More than 70% of pregnant women were younger than 34 years old, while 71.04% of women became pregnant through ART were older than 35 years old in 2017 (**Table 1**). The percentage of twin pregnancy was higher in ART conceptions than that in natural pregnancy, but the rates of twin birth in ART decrease slightly during the study period (**Supplement Figure 2**).

TABLE 1 | Maternal age among total pregnancies and ART conceptions in 2017.

Maternal age	*Total pregnancies ($N = 195{,}113$)		ART conceptions (cycles = 37,849)	
	No.	%	No.	%
≤24	18,965	9.71	129	0.3
25–34	119,496	61.23	10,830	28.6
≥35	56,652	29.03	26,890	71.04

*The maternal age of natural pregnancies can't be obtained.

ART and Birth Weight

A total of 1,405,625 natural pregnancy births and 50,988 ART conception births from 2011 to 2017 were included in the analysis. The proportions of very low birth weight (4.12% vs. 0.76%, $p < 0.001$) and low birth weight (32.09% vs. 7.05%, $p < 0.001$) were significantly higher in ART conception than in natural pregnancy (**Table 2**). The differences in very low birth weight (1.94% vs. 0.76%, $p < 0.001$) and low birth weight (9.46% vs. 7.05%, $p < 0.001$) still hold true when the analyses were restricted to singletons only (**Table 2**). The annual proportions of very low birth weight and low birth weight in ART conception, ART conception singletons and natural pregnancy are in the **Supplement Table 1**.

ART and Preterm Birth

A total of 611,200 natural pregnancy births and 22,078 ART conception births from 2014 to 2016 were included in the analysis. The preterm birth rate (<37 weeks) of ART conception was significantly higher than that of natural pregnancy ($p < 0.001$) (**Table 3**). The differences hold true when the analyses were restricted to singletons (**Table 3**). It is important to note, that rate of preterm birth in ART conception is higher in younger mothers (**Table 3**). The annual proportions of preterm births in ART conception, ART conception singletons and natural pregnancy are in the **Supplement Table 2**.

ART and Birth Defects

Infants born from 2014 to 2016 were included in this analysis. Children conceived by ART, compared to those through natural pregnancy, had an increased likelihood of developing any defects (0.83% vs. 0.42%, $p < 0.05$) (**Table 4**), cardiovascular defects (0.24% vs. 0.06%, $p < 0.01$) (item 1), central nervous system defects (0.06% vs. 0.01%, $p < 0.05$) (item 2), and gastrointestinal defects (0.09% vs. 0.03%, $p < 0.05$) (item 3). On the contrary, the percentage of urogenital, musculoskeletal, ear and facial defects and chromosome abnormality (items 4–7) were lower in children conceived by ART, probably because prenatal diagnoses and artificial abortions were routinely employed after ART conceptions. The annual proportions of birth defects in ART conception and natural pregnancy are in the **Supplement Tables 3, 4**.

TABLE 2 | Proportion of very low birth weight (VLBW) and low birth weight (LBW) in natural pregnancy, ART conception and ART conception singleton in 2011–2017.

Birthweight (g)	Natural pregnancy (*N* = 1,405,625)		ART conception (*N* = 50,988)		*P-value*
	No.	%	No.	%	
VLBW (<1,500)	10,770	0.76	2,103	4.12	<0.001
LBW(1,500–2,499)	99,111	7.05	16,363	32.09	<0.001
Birthweight (g)	**Natural pregnancy (*N* = 1,405,625)**		**ART conception singleton (*N* = 28,455)**		***P-value***
	No.	**%**	**No.**	**%**	
VLBW (<1,500)	10,770	0.76	554	1.94	<0.001
LBW (1,500–2,499)	99,111	7.05	2,693	9.46	<0.001

TABLE 3 | Proportion of preterm birth (<37 weeks) in natural pregnancy, ART conception and ART conception singleton in 2014–2016.

Maternal age	Natural pregnancy (*N* = 611,200)		ART conception (*N* = 22,078)		*P-value*
	No.	%	No.	%	
≤24	5,002	8.3	53	48.6	<0.001
25–34	31,159	7.7	3,960	36.8	<0.001
≥35	15,348	10.4	3,756	33.5	<0.001
Maternal age	**Natural pregnancy (*N* = 611,200)**		**ART conception (*N* = 12,804)**		***P-value***
	No.	**%**	**No.**	**%**	
≤24	5,002	8.3	10	16.1	<0.05
25–34	31,159	7.7	650	11.3	<0.02
≥35	15,348	10.4	961	13.7	<0.01

DISCUSSION

By reviewing the data of the recent cohort of ART-conceived children in Taiwan, we observed an increased occurrences of low birth weight, preterm birth, and birth defects. This study is unique that we analyzed a fairly large number (more than 50,000) of children who were conceived by ART during a recent period of time (from 2011 to 2017). Therefore, we were able to obtain highly significant results and to compare the prevalence of birth defects between the two groups. It is disappointing that complications still occurred in a country where outstanding ART technologies and modern medical care are in place.

Both embryo manipulation and environmental factors within the laboratory appear to cause epigenetic changes during the

TABLE 4 | Prevalence of birth defects in Natural pregnancy and ART conception in 2014–2016.

*Birth defects	Natural pregnancy (*N* = 611,200)		ART conception (*N* = 22,078)		*P*-value
	No.	%	No.	%	
Any defects	2,572	0.42	201	0.83	<0.05
1	385	0.06	58	0.24	<0.01
2	59	0.01	15	0.06	<0.05
3	205	0.03	22	0.09	<0.05
4	315	0.05	20	0.08	0.216
5	727	0.12	11	0.05	<0.001
6	724	0.12	7	0.03	<0.001
7	30	0.02	2	0.03	0.165

**The labels of the defects: cardiovascular (1), central nervous system (2), gastrointestinal (3), urogenital (4), musculoskeletal (5), ear and facial defects (6), and chromosome abnormalities (7).*

first stages of embryo development (19, 20). Alterations of gene expression occur (21), and these modifications can be heritable (22). The altered methylations of the endothelial nitric oxide synthase (eNOS) gene leads to decreased vascular NO synthesis in the aorta, and endothelial dysfunction and increased stiffness lead to arterial hypertension (23, 24). Exogenous Gonadotropins administered to the ovary during controlled ovarian stimulation causes epigenetic changes in peg1, kcnq1ot1, zac (Zac1), and h19 (25). *Zac1* plays an important role in neural stem cell quiescence, proliferation and differentiation (26). Either loss or gain of *Zac1* expression was reported to be associated with reduced growth rates and intellectual disability (27), and altered methylation of kcnq1ot1 and h19 was also reported in children with intellectual disability (28). Epigenetic impairments of DNA methylation were also related to pediatric pre-B cell acute lymphoblastic leukemia (29).

One limitation of the current study, similar to that of all these kinds of studies, is being unable to exclude the intrinsic risks of couples using ART, including the classic factors related to low birth weight, preterm birth, and birth defects. Generally, women undergoing ART are older and more often primiparous than the general obstetric population. Consequently, these mothers carry additional age and parity-related risks (30–32). Furthermore, the prevalence of chromosome abnormalities could not be accurately assessed in children conceived by ART because prenatal genetic diagnoses (PGD) and artificial abortions were routinely applied if the patients were desired while ART conceptions was performed in Taiwan.

CONCLUSION

Taiwan has been well-known for its mature and outstanding ART technologies and modern medical care. However, the risks for children conceived by ART during 2011–2017 appeared to have increased, which suggests that the risks are more than a statistic result. Rather, it presents itself a real phenomenon that may or may not be avoided in ART procedures.

Thus, in order to reduce the risks in ART-conceived children, the current ART policy and regulations may consider revising granting permissions for ART applications, including extending the policy to suitable applicants only. For example, to reduce age-related risk factors, ART permission should not be granted to women aged 50 and over. It is also high time to educate public about risks of using ART and raise social awareness on this issue.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

ETHICS STATEMENT

Ethical approval was obtained by the Ethics Committee of Taipei Hospital, Ministry of Health and Welfare (TH-IRB-0020-0002).

AUTHOR CONTRIBUTIONS

H-YC and W-LH investigated and supervised the findings of this work. C-HC and C-YH collected the data and analysis. WC conceived the present idea, wrote the manuscript, and takes primary responsibility for communication with the journal and editorial office during the submission process, throughout peer review and during publication.

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SUPPLEMENTARY MATERIAL

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REFERENCES

1. Hsu JC, Su YC, Tang BY, Lu CY. Use of assisted reproductive technologies before and after the Artificial Reproduction Act in Taiwan. *PLoS ONE*. (2018) 13:e0206208. doi: 10.1371/journal.pone.0206208
2. Fernando D, Halliday JL, Breheny S, Healy DL. Outcomes of singleton births after blastocyst versus nonblastocyst transfer in assisted reproductive technology. *Fertil Steril*. (2012) 97:579–84. doi: 10.1016/j.fertnstert.2011.12.032
3. Hayashi M, Nakai A, Satoh S, Matsuda Y. Adverse obstetric and perinatal outcomes of singleton pregnancies may be related to maternal factors associated with infertility rather than the type of assisted reproductive technology procedure used. *Fertil Steril*. (2012) 98:922–8. doi: 10.1016/j.fertnstert.2012.05.049
4. D'Angelo DV, Whitehead N, Helms K, Barfield W, Ahluwalia IB. Birth outcomes of intended pregnancies among women who used assisted reproductive technology, ovulation stimulation, or no treatment. *Fertil Steril*. (2011) 96:314–20 e2. doi: 10.1016/j.fertnstert.2011.05.073
5. Shih W, Rushford DD, Bourne H, Garrett C, McBain JC, Healy DL, et al. Factors affecting low birthweight after assisted reproduction technology: difference between transfer of fresh and cryopreserved embryos suggests an adverse effect of oocyte collection. *Hum Reprod*. (2008) 23:1644–53. doi: 10.1093/humrep/den150
6. Kalra SK. Adverse perinatal outcome and *in vitro* fertilization singleton pregnancies: what lies beneath? Further evidence to support an underlying role of the modifiable hormonal milieu in *in vitro* fertilization stimulation. *Fertil Steril*. (2012) 97:1295–6. doi: 10.1016/j.fertnstert.2012.03.047
7. Zhu L, Zhang Y, Liu Y, Zhang R, Wu Y, Huang Y, et al. Maternal and live-birth outcomes of pregnancies following assisted reproductive technology: a retrospective cohort study. *Sci Rep*. (2016) 6:35141. doi: 10.1038/srep35141
8. Tan SL, Doyle P, Campbell S, Beral V, Rizk B, Brinsden P, et al. Obstetric outcome of *in vitro* fertilization pregnancies compared with

normally conceived pregnancies. *Am J Obstet Gynecol*. (1992) 167:778–84. doi: 10.1016/S0002-9378(11)91589-0
9. Fechner AJ, Brown KR, Onwubalili N, Jindal SK, Weiss G, Goldsmith LT, et al. Effect of single embryo transfer on the risk of preterm birth associated with *in vitro* fertilization. *J Assist Reprod Genet*. (2015) 32:221–4. doi: 10.1007/s10815-014-0381-2
10. Hansen M, Greenop KR, Bourke J, Baynam G, Hart RJ, Leonard H. Intellectual disability in children conceived using assisted reproductive technology. *Pediatrics*. (2018) 142:e20181269. doi: 10.1542/peds.2018-1269
11. Sandin S, Nygren KG, Iliadou A, Hultman CM, Reichenberg A. Autism and mental retardation among offspring born after *in vitro* fertilization. *JAMA*. (2013) 310:75–84. doi: 10.1001/jama.2013.7222
12. Fountain C, Zhang Y, Kissin DM, Schieve LA, Jamieson DJ, Rice C, et al. Association between assisted reproductive technology conception and autism in California, 1997–2007. *Am J Public Health*. (2015) 105:963–71. doi: 10.2105/AJPH.2014.302383
13. Meister TA, Rimoldi SF, Soria R, von Arx R, Messerli FH, Sartori C, et al. Association of assisted reproductive technologies with arterial hypertension during adolescence. *J Am Coll Cardiol*. (2018) 72:1267–74. doi: 10.1016/j.jacc.2018.06.060
14. Kuiper D, Hoek A, la Bastide-van Gemert S, Seggers J, Mulder DJ, Haadsma M, et al. Cardiovascular health of 9-year-old IVF offspring: no association with ovarian hyperstimulation and the *in vitro* procedure. *Hum Reprod*. (2017) 32:2540–8. doi: 10.1093/humrep/dex323
15. Valenzuela-Alcaraz B, Crispi F, Bijnens B, Cruz-Lemini M, Creus M, Sitges M, et al. Assisted reproductive technologies are associated with cardiovascular remodeling *in utero* that persists postnatally. *Circulation*. (2013) 128:1442–50. doi: 10.1161/CIRCULATIONAHA.113.002428
16. Kallen B, Finnstrom O, Lindam A, Nilsson E, Nygren KG, Olausson PO. Cancer risk in children and young adults conceived by *in vitro* fertilization. *Pediatrics*. (2010) 126:270–6. doi: 10.1542/peds.2009-3225
17. Hargreave M, Jensen A, Toender A, Andersen KK, Kjaer SK. Fertility treatment and childhood cancer risk: a systematic meta-analysis. *Fertil Steril*. (2013) 100:150–61. doi: 10.1016/j.fertnstert.2013.03.017
18. Reigstad MM, Larsen IK, Myklebust TA, Robsahm TE, Oldereid NB, Brinton LA, et al. Risk of cancer in children conceived by assisted reproductive technology. *Pediatrics*. (2016) 137:e20152061. doi: 10.1542/peds.2015-2061
19. Rivera RM, Stein P, Weaver JR, Mager J, Schultz RM, Bartolomei MS. Manipulations of mouse embryos prior to implantation result in aberrant expression of imprinted genes on day 9.5 of development. *Hum Mol Genet*. (2008) 17:1–14. doi: 10.1093/hmg/ddm280
20. Gomes MV, Huber J, Ferriani RA, Amaral Neto AM, Ramos ES. Abnormal methylation at the KvDMR1 imprinting control region in clinically normal children conceived by assisted reproductive technologies. *Mol Hum Reprod*. (2009) 15:471–7. doi: 10.1093/molehr/gap038
21. Lucas E. Epigenetic effects on the embryo as a result of periconceptional environment and assisted reproduction technology. *Reprod Biomed Online*. (2013) 27:477–85. doi: 10.1016/j.rbmo.2013.06.003
22. Chen H, Zhang L, Deng T, Zou P, Wang Y, Quan F, et al. Effects of oocyte vitrification on epigenetic status in early bovine embryos. *Theriogenology*. (2016) 86:868–78. doi: 10.1016/j.theriogenology.2016.03.008
23. Rexhaj E, Paoloni-Giacobino A, Rimoldi SF, Fuster DG, Anderegg M, Somm E, et al. Mice generated by *in vitro* fertilization exhibit vascular dysfunction and shortened life span. *J Clin Invest*. (2013) 123:5052–60. doi: 10.1172/JCI68943
24. Stoll S, Wang C, Qiu H. DNA Methylation and Histone Modification in Hypertension. *Int J Mol Sci*. (2018) 19:1174. doi: 10.3390/ijms19041174
25. Ventura-Junca P, Irarrazaval I, Rolle AJ, Gutierrez JI, Moreno RD, Santos MJ. *In vitro* fertilization (IVF) in mammals: epigenetic and developmental alterations. Scientific and bioethical implications for IVF in humans. *Biol Res*. (2015) 48:68. doi: 10.1186/s40659-015-0059-y
26. Daniel G, Schmidt-Edelkraut U, Spengler D, Hoffmann A. Imprinted Zac1 in neural stem cells. *World J Stem Cells*. (2015) 7:300–14. doi: 10.4252/wjsc.v7.i2.300
27. Adnani L, Langevin LM, Gautier E, Dixit R, Parsons K, Li S, et al. Zac1 Regulates the differentiation and migration of neocortical neurons via Pac1. *J Neurosci*. (2015) 35:13430–47. doi: 10.1523/JNEUROSCI.0777-15.2015
28. Mayo S, Monfort S, Rosello M, Oltra S, Orellana C, Martinez F. In pursuit of new imprinting syndromes by epimutation screening in idiopathic neurodevelopmental disorder patients. *Biomed Res Int*. (2015) 2015:341986. doi: 10.1155/2015/341986
29. Wong NC, Ashley D, Chatterton Z, Parkinson-Bates M, Ng HK, Halemba MS, et al. A distinct DNA methylation signature defines pediatric pre-B cell acute lymphoblastic leukemia. *Epigenetics*. (2012) 7:535–41. doi: 10.4161/epi.20193
30. Kessler I, Lancet M, Borenstein R, Steinmetz A. The problem of the older primipara. *Obstet Gynecol*. (1980) 56:165–9.
31. Prysak M, Lorenz RP, Kisly A. Pregnancy outcome in nulliparous women 35 years and older. *Obstet Gynecol*. (1995) 85:65–70. doi: 10.1016/0029-7844(94)00330-G
32. Ziadeh SM. Maternal and perinatal outcome in nulliparous women aged 35 and older. *Gynecol Obstet Invest*. (2002) 54:6–10. doi: 10.1159/000064689

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Identification of High-Risk Pregnancies in a Remote Setting Using Ambulatory Blood Pressure: The MINDI Cohort

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Background: Ambulatory blood pressure is a potential tool for early detection of complications during pregnancy, but its utility in impoverished settings has not been assessed. This cross-sectional study aimed to determine whether maternal infections, nutrient deficiencies and inflammation (MINDI) were associated with four measures of maternal blood pressure (BP) and to determine their association with symphysis-fundal-height (SFH).

Methods: Environmental and dietary factors, intake of iron and a multiple-nutrient supplement (MNS), markers of inflammation, protein, anemia, folate, vitamins B_{12}, A and D status, and urogenital, skin, oral and intestinal nematode infections were measured in indigenous pregnant Panamanian women. Stepwise multiple linear and logistic regression models explored determinants of systolic and diastolic blood pressure (SBP, DBP), hypotension (SBP $<$ 100 and DBP $<$ 60), mean arterial pressure (MAP), elevated MAP (eMAP), and pulse pressure (PP). Associations of BP with intestinal nematodes and with SFH Z scores ($\geq$16 wk) were also explored.

Results: Despite absence of high SBP or DBP, 11.2% of women had eMAP. Furthermore, 24.1% had hypotension. Linear regression showed that hookworm infection was associated with higher SBP ($P = 0.049$), DBP ($P = 0.046$), and MAP ($P = 0.016$), whereas Ascaris was associated with lower DBP ($P = 0.018$) and MAP ($P = 0.028$). *Trichomonas* was also associated with lower SBP ($P < 0.0001$) and MAP ($P = 0.009$). The presence of *Trichuris* (OR: 6.7, 95% CI 1.0–44.5) and folic acid deficiency (OR: 6.9, 95% CI 1.4–33.8) were associated with increased odds of eMAP. The odds of low BP was higher in the presence of *Ascaris* (OR: 3.63 $\pm$ 2.28, $P = 0.040$), but odds were lowered by MNS (OR: 0.35 $\pm$ 0.11, $P = 0.001$), more intake of animal-source foods/wk (OR: 0.7, 95% CI 0.5–0.9) and by higher concentrations of IL-17 (OR: 0.87 $\pm$ 0.05, $P = 0.016$).

Conclusion: MINDI were bi-directionally associated with blood pressure indicators. In this MINDI cohort, infections, nutrients and cytokines both raised, and lowered BP indices. The presence of eMAP identified pregnant women at risk of hypertension whereas low PP was associated with lower SFH. Therefore, MAP and PP may help in detecting women at risk of adverse pregnancy outcomes in settings with limited access to technology.

Keywords: MAP, pulse pressure, symphysis-fundal height, micronutrients, protein status, uro-genital infections, nematodes

INTRODUCTION

Appropriate pregnancy follow-up is a major challenge in settings where access to health care is limited. Fortunately, maternal blood pressure (BP) is easily measured even in remote settings. It is considered as an indicator of quality antenatal care (1) and useful in predicting hypertensive disorders of pregnancy (HDPs), preterm-birth and small-for-gestational-age (SGA) infants (2). In addition to systolic and diastolic blood pressure (SBP and DBP), both mean arterial pressure (MAP), and pulse pressure (PP) (3) also have clinical value. SBP, DBP (4), MAP (5), and PP (6, 7) are known to be elevated in early pregnancy before the development of HDPs whereas low DBP and MAP have been associated with poor fetal outcomes (8, 9). However, clinical interpretation may be misleading if co-existing conditions such as multiple infections, nutritional deficiencies, and inflammation (MINDI) modulate the relationship between blood pressure and pregnancy outcomes, including poor intrauterine growth.

Maternal nutrient deficiencies have been associated with both abnormal maternal blood pressure and decreased fetal growth. Maternal protein-energy malnutrition can lead to intra-uterine growth retardation (IUGR) (10), and low protein intake (<65 g/d) more than tripled the risk of HDPs (11). Although severe anemia is one of the main factors associated with HDPs (12), elevated hemoglobin (≥132 g/L) was positively associated with SBP and DBP (13), and hematocrit was positively correlated with MAP and with peripheral vascular resistance (14). With respect to vitamins, increase in vitamin D concentration by ≥ 30 nmol/L from the first to third trimester lowered the odds of preeclampsia (15). Homocysteine, which increases in response to low folate or vitamin B_{12} (16), was positively associated with MAP during pregnancy (17), and has been implicated in the physiopathology of HDPs and IUGR (16). Studies on vitamin A are contradictory. Women with retinol concentration >1.08 μmol/L had decreased risk of HDPs in Peru (18) whereas retinol concentrations > 1.05 μmol/L increased the risk of HDPs in Zimbabwe (19). Together, these studies highlight the potential associations of nutrients with blood pressure, but no studies have explored the combined impact of co-existing nutrient deficiencies on maternal/fetal health.

The limited evidence to date shows that common infections in impoverished rural populations affect blood pressure and pregnancy outcomes in a pathogen-specific manner. A prospective-cohort study showed that acute malaria lowered SBP, DBP and MAP but not PP (20) whereas both urinary infections (12) and the protozoan tissue parasite, *Toxoplasma gondii* (21) have been shown to increase odds of hypertension in pregnancy. The nature of the relationship between infection and blood pressure may depend on whether the infection induces a pro- or anti-inflammatory response. Inflammation modulates blood pressure in pregnancy (22) as evidenced by the association of HDPs with inflammatory markers including C-reactive protein (CRP) (23, 24) and with two pro-inflammatory cytokines, interleukin (IL)-6 and tumor necrosis factor (TNF)-α (25). In contrast, down-regulation of the pro-inflammatory response by IL-10 and T-regulatory cells (T-regs) has been shown to reduce the pathology of HDPs (26).

In areas where infections and nutrient deficiencies are common, a direct association between maternal blood pressure and fetal growth is difficult because of the absence of ultrasound, the current gold standard for fetal biometry assessment (27). However, SFH is widely used in Latin America for estimating fetal growth using PAHO standards (28). New standards for symphysis-fundal height (SFH) according to gestational age (GA) were recently developed by the INTERGROWTH project based on a large database of pregnant women from Brazil, China, India, Italy, Kenya, Oman, United Kingdom, and United States (29). The INTERGROWTH Project now allows SFH to be used as a first level screening tool for assessing the feto-maternal unit when ultrasound is not available. Currently there is no information on the application of new INTERGROWTH standards in Latin America.

In collaboration with the Panamanian Ministry of Health, we had previously collected a large dataset from our MINDI cohort of indigenous pregnant women in a region where HDPs and low birthweight are major public health concerns (30). Since BP measurements have been used in the early prediction of HDPs (31) and SFH has shown a sensitivity of 52% and a specificity of 92% in the detection of IUGR (28), and given that both BP and SFH, were the only clinical tools available to detect women at risk of adverse pregnancy outcomes, a comprehensive analysis of the database was used

Abbreviations: BP, blood pressure; CRP, C-reactive protein; DBP, diastolic blood pressure; eMAP, elevated mean arterial pressure; GA, gestational age; HDPs, hypertensive disorders of pregnancy; IFN, Interferon; IL, interleukin; IUGR, intra-uterine growth retardation; MAP, mean arterial pressure; MNS, multiple nutrient supplement; NLR, neutrophil/lymphocyte ratio; PP, pulse pressure; RBC, red blood cells; RBP, retinol-binding protein; SBP, systolic blood pressure; SFH, symphysis-fundal height; SGA, small-for-gestational-age; TNF, tumor necrosis factor; USG, urinary specific gravity; WBC, white blood cells.

to identify associations among BP measurements, MINDI, and the recently released INTERGROWTH standards for symphysis-fundal height (29). The objectives of this cross-sectional study using our MINDI cohort were to determine if multiple infections, nutrient deficiencies and inflammation were associated with measures of maternal blood pressure [SBP, DBP, MAP, PP, elevated MAP (eMAP), and low blood pressure (<100/60 mmHg)] and to determine which BP measurements were associated with SFH using the new INTERGROWTH standards. We also investigated associations of parasitic infections, in particular intestinal nematodes with BP measurements, given previous bidirectional associations of intestinal nematodes with CRP during pregnancy in this same population (32).

METHODS

Study Population

This cross-sectional study was conducted between August and December 2010 in the extremely impoverished Ngäbe-Buglé indigenous population in western Panama. In Panama, 13% of maternal mortality has been attributed to HDPs (30). Based on government statistics, 6.7% of institutional deliveries are low birthweight infants at the country level (33), whereas low birthweight rates are as high as 14% in the Ngäbe population (34). We have previously reported that pregnant women in our study had a high prevalence (>50%) of several vaginal and intestinal infections as well as oral, skin and urinary tract infections (35). Despite distribution of iron and micronutrient supplements by the Ministry of Health, >40% of these women had deficiencies of vitamins A, D and B_{12}, and CRP was elevated in 16.4% of these women (32). In the study area ∼40% of deliveries occurred at home, the zone was not endemic for malaria. Women were considered for the study if consulting for normal pregnancy follow up, and they did not present with signs of distress. The presence of HIV and gestational diabetes had been ruled-out by the Ministry of Health (35).

Ethics and Recruitment

This study was carried out in accordance with the recommendations of the Operational Guides of Bioethics in Research, of the Gorgas Memorial Institute Research Bioethics Committee (Panama). Ethics approval was obtained from the Gorgas Memorial Institute and from McGill University. Participants gave written informed consent in accordance with the Declaration of Helsinki.

Indigenous women had government-driven incentives to attend pregnancy follow-up. The Panamanian Ministry of Health ran a program of food vouchers that were given to pregnant women when they attended pregnancy follow-up at the local health center. All pregnant women attending normal pregnancy follow-up in 14 health centers of the Ngäbe-Buglé region in Chiriquí, Panama were invited to participate. Most women lived within 2 h walking-distance from community health centers. For the study, nurses working at health centers informed the community of the visit of the research team during scheduled pregnancy follow-up days, and that they could get a laboratory evaluation without needing to travel to the regional hospital, located 1–3 h by car. Based on the annual estimate of 2,127 live-births in the Ngäbe-Buglé community in 2010 (33), we recruited >90% of the pregnant women in the region at the time of the study. Given the prevalence of 4.5% for HDPs in a population of women ≤20 y from Panama (36), and on successful use of ambulatory blood pressure to detect a prevalence of 4.7% HDPs (37), we estimated that a sample size of 67 pregnant women would allow us to detect women at risk of HDPs. Based on 6.7% prevalence of low birthweight (33), our proxy for impaired fetal growth, we estimated that 92 pregnant women would be sufficient to detect small SFH with a level of confidence of 95%. We were able to recruit 213 pregnant women, 174 of whom were beyond 16 wk of pregnancy, the minimal GA for SFH to be compared with the INTERGROWTH standards (29).

Questionnaire and Physical Examination

Pregnant women answered questions about reproductive history, daily intake of iron supplements (60 mg tablets), multiple nutrient supplements (MNS) (tbsp/d), weekly intake of animal-source foods, fruits and vegetables, field work (hrs/d), and wood smoke exposure (h/d). Anthropometry was measured and the body mass index (BMI) was classified as underweight, normal or overweight using Pan American Health Organization standards for GA in pregnant women (28). When used as a continuous variable, maternal BMI was calculated as maternal weight/height^2.

Maternal blood pressure (Omron HEM-790IT® automatic BP monitor) was measured in a sitting position, and re-measured if elevated (SBP ≥ 140 or DBP ≥ 90 mmHg), in which case the second measurement was recorded (38, 39). As most women arrived to health centers after considerable walking, at least 15 min rest were allowed before examination. Intake of coffee and wood smoke were recorded. URISCAN® dip-stick strips on a Miditron-M semi-automated reflectance photometer were used for semi-quantitative measurements of urinary specific gravity (USG) and protein. Hypertension was defined as a combination of elevated SBP and DBP. Mothers were considered to have HDPs if they presented with hypertension and dipstick-proteinuria ≥ 1+ or if symptoms of preeclampsia were present after week 20 of pregnancy (40). Low blood pressure was defined as SBP <100 and DBP <60 mmHg, corresponding to values <10th centile of SBP and DBP from a large cohort of pregnant women (n = 10,327) (2). MAP was calculated as DBP + 1/3 (SBP-DBP) (31). Trimester-specific cutoffs for elevated MAP (eMAP) in pregnancy were >87 mmHg (10–18 wks), >84 mmHg (18–34 wks), and >86 mmHg (after 34 wks) (41), and low MAP was defined as <70 mmHg (42). Pulse pressure was calculated as the difference between SDP and DBP (3). Cut-offs for elevated PP (>68 mmHg) and low PP (<42 mmHg) were based on a large population study of normal pregnant women (43).

SFH was measured after the mother had recently emptied her bladder and while she was in a supine position. A non-elastic tape was placed at the upper border of the symphysis pubic bone, and straightened over the uterus until reaching the fundus. The cubital edge of the hand was used to hold the tape at the point of the fundus while it was turned to see the numbers; the value was recorded to the nearest complete half

centimeter. The INTERGROWTH standards for SFH were used to calculate SFH Z-scores and centiles in women with GA ≥ 16 weeks ($n = 177$) (29).

Infections

Maternal infections were evaluated both clinically and using laboratory tests as previously described (35). Briefly, skin lesions compatible with scabies and oral caries were detected during the clinical exam (0 = no, 1 = yes), and bacteriuria based on microscopic analysis of centrifuged urine was scored 0 (absent), 1+ (few), 2+ (moderate) and 3+ (abundant). Vaginal Gram smears were assigned semi-quantitative scores (0–4) for *Lactobacillus, Bacteroides/Gardnerella* and *Mobiluncus*, and these scores were used to diagnose bacterial vaginosis (BV) based on a Nugent score ≥7, calculated as *Bacteroides/Gardnerella* score + (4 – *Lactobacillus* score) + (*Mobiluncus* score/2) (44). Semi-quantitative scores were similarly assigned for Gram-detected vaginal trichomoniasis and diplococcal infection, and for vaginal yeast detected by direct smears. Presence of intestinal nematodes (*Ascaris*, hookworm and *Trichuris*) was identified using direct microscopic fecal examination, Kato-Katz and Flotac® from the 120 women who provided stool samples, as previously described (35).

Laboratory Analyses

Hematological Status and Inflammation

Complete red (RBC) and white blood cell (WBC) counts (BC-5500 Mindray Auto Hematology Analyzer) were performed. Anemia was defined as hemoglobin <110 g/L (45). Hematocrit was compared against normal ranges for the first (31–41%), second (30–39%), and third (28–40%) trimesters (46). To estimate the degree of hemoconcentration, hematocrit was classified in quantiles according to our population distribution: <25th quantile, 25–50th, 50–75th, and >75th quantiles (<33.2%, 33.2–35%, 35–36.9%, and >36.9%, respectively). Hypohydration was assessed as USG > 1,020 (47). Serum was analyzed for CRP using solid phase ELISA (MP Biomedicals, Orangeburg, NY) with a minimum detectable concentration of 0.95 nmol/L. Nine serum cytokines, IL-1β, IL-4, IL-6, IL-10, IL-12, IL-13, IL-17, interferon (IFN)-γ and TNF-α, were quantified by Luminex using a Human Cytokine/Chemokine Magnetic Bead Panel (Millipore Corporation Canada).

Maternal Nutritional Status

Serum samples were processed for folic acid and vitamin B_{12} concentrations using immunoelectro-chemiluminescence (MODULAR E170, Roche Diagnostics GmBH, Mannheim, Germany); for 25-OH vitamin D using the LIAISON, DiaSorin direct competitive chemiluminescence immunoassay; for vitamin A using HPLC (48); and for retinol binding protein (RBP) using Human RBP4 ELISA (MP Biomedicals) with a standard curve range between 0.14 and 100 ng/mL. Folic acid deficiency was defined as <10 nmol/L and vitamin B_{12} deficiency as <150 pmol/L (49). We used a cutoff for vitamin D deficiency of <50 nmol/L (50). Low vitamin A was defined as <1.05 μmol/L (51, 52) and low protein status as RBP <30 mg/L (53). Some women provided a sample of their coffee ($n = 28$), where caffeine was measured using HPLC (54).

Statistical Analysis

All statistical analyses were performed using STATA 14 (StataCorp, TX, USA). Given that a few women did not provide urine ($n = 5$) or vaginal samples ($n = 2$), that the volume of serum samples was insufficient to process vitamin A analysis ($n = 3$) and cytokine assays ($n = 1$), and that only 120 women provided stool samples for intestinal nematode screening, multiple linear regression models included the STATA complete-case analysis function (55) which allowed us to both maximize the sample size of each final model and confirm the randomness of missing data using Little's chi-squared test (56).

Initial exploratory models for SBP, DBP, MAP, and PP were developed using stepwise multiple linear regressions accepting variables with $P < 0.15$ for six distinct clusters of variables: (1) environmental/supplementation variables (intake of animal-source foods, fruits and vegetables, coffee, intake of iron and MNS, field work, wood smoke); (2) RBC indices including hematocrit quantile and anemia; (3) inflammation indicators [WBC count and differential, neutrophil-lymphocyte ratio (NLR), CRP and cytokines]; (4) nutritional deficiencies (low protein and deficiencies of folic acid, vitamins B_{12}, A and D); (5) infections with prevalence ≥10% (caries, scabies, and BV) as well as semi-quantitative scores for urinary bacteria, and vaginal *Bacteroides/Gardnerella*, *Mobiluncus*, *Lactobacillus,* trichomoniasis, diplococci and yeast; and (6) intestinal nematode infections (*Ascaris*, hookworm and *Trichuris*). We controlled for gestational age in all models. Maternal characteristics (age, parity, BMI category and urinary gravity ≤1,020) were also included if they had $P < 0.15$ in screening models.

Spearman correlations were calculated among independent variables within each set to avoid inclusion of significantly correlated variables in the same regression model. The independent occurrence of physiologically-related variables (low RBP with vitamin A deficiency and elevated CRP; anemia with low hematocrit) was tested using Chi^2 analysis. Then, final stepwise multiple linear regression models were developed. Depending on the sample size for each final model, inclusion of 6–10 independent variables allowed us to have power of 0.80 and a medium effect size (57). If the stepwise process yielded more variables than permitted in the regression model given the sample size, the filter was lowered from 0.15 to $P < 0.10$ or $P < 0.05$ until the number of variables was appropriate for the sample size.

Linear regression models for SBP, DBP, MAP, and PP were run with ($n = 120$) and without ($n = 213$) intestinal nematodes. Given that MAP (but not other BP measurements) differed by trimester, multiple linear regressions were also run for MAP by trimester, with and without intestinal nematodes. We also confirmed the absence of collinearity based on a variance inflation factor (VIF) <10 and the stability of regression coefficients by a condition number <30. Significance of variables in the final models was set at $P < 0.05$. Only the final models are presented.

Variables associated with eMAP and low blood pressure (SBP <100 and DBP<60 mmHg) were explored using multiple logistic regression analysis, following the same sequence of steps as used for the multiple linear regressions.

For SFH Z-scores, Student's *T*-tests were used to establish differences between the presence/absence of abnormal blood pressures: eMAP (classified by GA), low MAP (<70 mmHg), low BP (<100/60 mmHg), and low PP (<42 mmHg). For assessing if SFH Z-score was associated with BP indicators, independent factor linear regression analyses were conducted. For each blood pressure indicator, the data were separated into three "factors" (factor 1: <10th centile; factor 2: 10–90th centile; factor 3: >90th centile) as determined by 10 and 90th centile values from our data (SBP, 90 and 117.6 mmHg; DBP, 51.4 and 72 mmHg; MAP, 65.6 and 86.6 mmHg; and PP, 30 and 51.6 mmHg). Regression models for SFH-Z-score were constructed using factor 2 as the reference factor. Reported coefficients compared women in factor 1 and in factor 3 with those in factor 2. Significance was set at $P < 0.05$. Associations were further tested after adjusting for two known risks for IUGR, low BMI and wood smoke exposure.

RESULTS

Population Characteristics

Descriptive data are reported in **Table 1**. The population had a series of risk factors for adverse pregnancy outcomes, including pregnancy in adolescents and those over 35 y, and primiparous and grand-multiparous (≥5) pregnancies. Most women used wood as fuel for cooking and 82% had access to <7 portions/wk of animal source foods. Coffee intake was common, but the caffeine content of coffee was low, with a median (min-max) of 4.5 (0.3–24.5) mg/100 mL. The majority of women (67.1%) had a normal weight-for-height-for-gestational age, but low weight for GA (9.9%) and overweight (23%) were also present. Over 50% of women had vitamin B_{12} or D deficiency, and over 20% had low RBP, low vitamin A or folic acid deficiency. RBP concentration was not correlated with concentration of vitamin A ($r_s = 0.04$, $P = 0.55$, $n = 212$) or CRP ($r_s = -0.10$, $P = 0.12$, $n = 212$), but was correlated with B_{12} ($r_s = 0.15$, $P < 0.03$, $n = 207$) which is predominantly found in animal source foods. Of the two supplementation programs in the communities, iron tablets reached over 75% of women, and half the women took MNS, although at a median intake well below the recommended 6 tbsp/d. Despite use of supplements, over one-third of the women were anemic. Infections were extremely common and 97% of women had at least two infections. The most prevalent were vaginal infections, followed by intestinal nematodes, urinary, oral and skin infections (**Table 1**).

In our population, 27% of the women had USG>1,020 indicating hypohydration and 27% had RBP<30 mg/L indicating low protein status and suggesting low oncotic pressure. Moreover, despite the high prevalence of anemia (38%), all women had normal RBC count and most had normal (93.4%) or high (2.3%) hematocrit, further supporting the presence of hypovolemia in our population and suggesting that the normal hemo-dilution of pregnancy is not occurring.

Trimester-specific blood pressure measurements are described in **Table 1**. None of the pregnant women had high SBP or DBP (**Figure 1**). SBP, DBP, and PP did not differ by trimester, but MAP did ($P = 0.035$).

Furthermore, none of the women presented with clinical manifestations of HDPs. Although urinary protein was found (2.9%), those women had urinary tract infection. However, using eMAP, risk of HDPs was detected in 11.3% women (**Figure 2**). With regard to PP, none of the women had elevated PP but 52.6% had low PP (**Figure 3**) and the 10th centile corresponded to a PP of 30 mmHg.

Based on the INTERGROWTH standards for SFH, 50.6% ($n = 88$) of women had SFH measurements below the 10th centile and 9.2% ($n = 16$) had measurements above the 90th centile (**Figure 4**).

Multiple Regression Models for Blood Pressure Measurements

Models including intestinal nematodes are shown in **Tables 2**, **3**. Models not including intestinal nematodes with a larger sample size are provided as **Tables S1, S2**.

Several infections emerged in our multiple regression models for SBP (**Table 2A**), DBP (**Table 2B**) and MAP (**Table 3A**), and depending on the infection, were associated with either higher or lower BP measurements. First, presence of scabies was associated with lower SBP, and presence of trichomoniasis with lower SBP and lower MAP. Second, it is important to highlight that, whereas hookworm or *Trichuris* were consistently associated with higher blood pressure measures [SBP, DBP, MAP, eMAP models (**Tables 2A,B**, **3A**, **4A**)], *Ascaris* was associated with lower blood pressure measures, DPB, MAP, and low blood pressure (**Tables 2B**, **3A**, **4B**). Furthermore, despite the much lower sample size, inclusion of intestinal nematode variables increased the adjusted R^2 of the final SBP, DBP and MAP models by more than 75% compared with models where intestinal nematode variables were not included (**Table S1**).

Regarding diet and nutrition, higher reported intake of MNS was associated with higher SBP (**Table 2A**), DBP (**Table 2B**), MAP (**Table 3A**), and PP (**Table 3B**), but was not associated with eMAP (**Table 4A**), whereas higher intake of animal-source foods was associated with decreased odds of BP < 100/60 (**Table 4B**). Also, two nutrient biomarkers were associated with BP: folic acid deficiency was associated with higher DBP and with increased odds of eMAP, and low RBP with higher DBP and MAP. Of note, folic acid deficiency was associated with higher MAP (**Table S1**), but lost its significance when intestinal nematodes were included (**Table 3A**).

Among inflammation indicators, two cytokines stood out in our models. TNF-α was associated with higher SBP (**Table 2A**), MAP and PP (**Tables 3A,B**); however, higher IL-17 was associated with lower PP, but only in the model without intestinal nematodes (**Table 3B**). Interestingly, IL-17 was not associated with SBP or DPB, but was associated with a lower likelihood of maternal BP<100/60 (**Table 4B**).

Although we controlled all our models for GA (wks), given that MAP differed by trimester, we ran predictive models by

TABLE 1 | Population characteristics in pregnant Ngäbe-Buglé women from rural Panama[a].

Maternal characteristics, *n* (%)		**Nutritional characteristics**	
Age		Weight for height category, *n* (%)	
≤19 yrs	62 (29.1%)	Underweight	21 (9.8%)
≥35 yrs	28 (13.1%)	Overweight	49 (23.0%)
Trimester		**Serum nutrients**	
First	26 (12.2%)	Retinol-binding protein	
Second	80 (37.6%)	[median (min-max)]	4.8 (0.3–41.2)
Third	107 (50.2%)	<30 mg/L, *n* (%)	57 (26.9%)
Parity		Vitamin B_{12}	
First gestation	60 (28.2%)	[median (min–max)]	100.0 (53.0–376.0)
≥5 gestations	69 (32.4%)	<150 pmol/L, *n* (%)	181 (84.9%)
Environment hazards		Vitamin D	
Wood smoke	195 (91.5%)	(mean ± SD)	44.5 ± 15.1
Fieldwork	110 (51.6%)	< 50 nmol/L, *n* (%)	138 (64.8%)
Blood pressure, (mean ± SD)		Vitamin A	
Systolic blood pressure, mmHg	102.8 ± 10.3	[median (min-max)]	1.2 (0.4–2.9)
First trimester	104.3 ± 10.3	<1.05 μmoll/L, *n* (%)	87 (41.4)
Second trimester	100.8 ± 10.9	Folic acid	
Third trimester	103.9 ± 9.7	[median (min-max)]	14.1 (6.3–45.4)
Diastolic blood pressure, mmHg	61.6 ± 8.5	<10 nmol/L, *n* (%)	51 (23.9%)
First trimester	63.3 ± 7.5	**Diet and supplementation, *n* (%)**	
Second trimester	59.8 ± 8.7	Animal source foods	
Third trimester	62.5 ± 8.5	<7 portions/wk	176 (82.6%)
Mean Arterial Pressure, mmHg		fruits-vegetables	
First trimester	77.6 ± 7.2	<7 portions/wk	179 (84.0%)
Second trimester	73.2 ± 8.5	Coffee intake	185 (86.8%)
Third trimester	76.3 ± 7.9	**Supplementation**	
Pulse pressure, mmHg		Iron, *n* (%)	163 (76.5%)
First trimester	40.7 ± 9.8	MNS, *n* (%)[2]	108 (50.7%)
Second trimester	41.0 ± 8.3	**Red blood cells**	
Third trimester	41.3 ± 8.5	Hemoglobin, g/L (mean ± SD)	111.6 ± 11.3
Infections, *n* (%)		Anemia, *n* (%)	81 (38.0%)
Caries	42 (19.7%)	Hematocrit (%) (mean ± SD)	34.8 ± 3.2
Scabies	37 (17.3%)	Mean corpuscular volume, fL (mean ± SD)	93.7 ± 6.0
Vaginal infections			
Trichomonas vaginalis	159 (75.3%)	**Urine analyses, *n* (%)**	
Bacterial vaginosis	128 (60.6%)	Urinary specific gravity > 1,020,	56 (26.9%)
Lactobacillus	113 (53.5%)	Bacteriuria ≥ 2+	54 (25.9%)
Bacteroides/Gardnerella	198 (93.8%)	**Intestinal parasites, *n* (%)[b]**	
Mobiluncus	174 (82.4%)	Hookworm	52 (56.6%)
Yeast	53 (24.9%)	*Ascaris*	39 (32.5%)
Diplococcus spp.	43 (20.3%)	*Trichuris*	15 (12.5%)

[a] *n = 208–213 for all, except for intestinal parasites (n = 120).*

[b] *MNS: Multiple nutrient supplement containing in every 100 g, energy (400 kcal), protein (12.0 g), lipids (12–14 g), vitamin A (250 μg), vitamin E (10 mg), vitamin B_1 (0.50 mg), vitamin B_2 (0.50 mg), vitamin B_3 (6.0 mg), vitamin B_6 (0.50 mg), vitamin B_{12} (0.90 μg), folic acid (85 μg), iron (4.0 mg iron bisglycinate), zinc (4.5 mg amino chelated), calcium (100 mg), phosphorus (55 mg) and copper (400 μg) (58).*

Means ± SD or frequencies (%) are presented[a].

trimester for MAP (**Table S3**). More cups/d of coffee were associated with lower MAP ($P = 0.040$) only in the first trimester, whereas higher Hb ($P = 0.003$) (model not shown), hematocrit ($P = 0.014$) (model not shown) as well as the red blood cell count ($P < 0.0001$) (**Table S3**) were associated with higher MAP also in the first trimester. In concordance, a higher hematocrit ($P = 0.003$) as well as hypohydration indicated by urinary gravity ≥ 1,020 ($P = 0.009$) were associated with higher MAP in the second trimester. In both the second ($P = 0.009$) and the third trimester ($P = 0.003$), MNS was associated with higher MAP and interestingly, *Ascaris* infection ($P = 0.006$) was associated with lower MAP but only in the third trimester (**Table S3**).

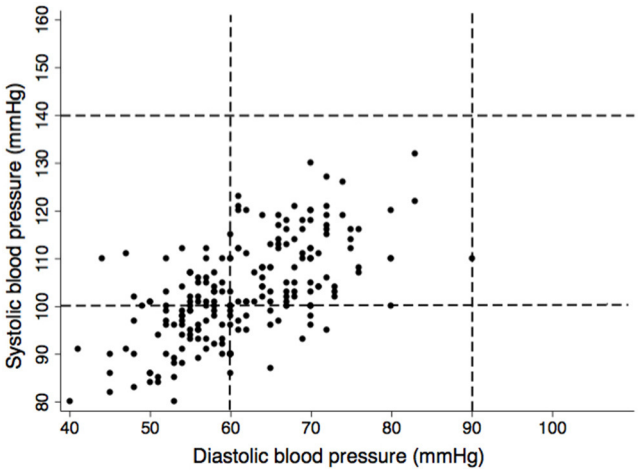

FIGURE 1 | Scatter plot of systolic vs. diastolic blood pressure in 213 pregnant Ngäbe-Buglé women from Panama. Dashed lines represent blood pressure limits for hypertension during pregnancy (≥140 mmHg for SBP and ≥90 mmHg for DBP) (31), lower limits were defined following the most conservative cut-offs (<100 mmHg for SBP and <60 mmHg) for pregnant women (2).

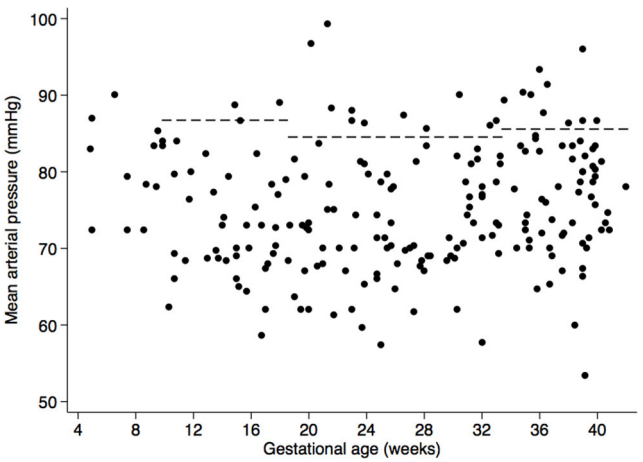

FIGURE 2 | Scatter plot of mean arterial pressure (MAP) according to gestational age. Dashed lines represent cutoffs for elevated MAP: >87 mmHg between weeks 10–18, >84 mmHg in weeks 18–34, and >86 mmHg after week 34 (41).

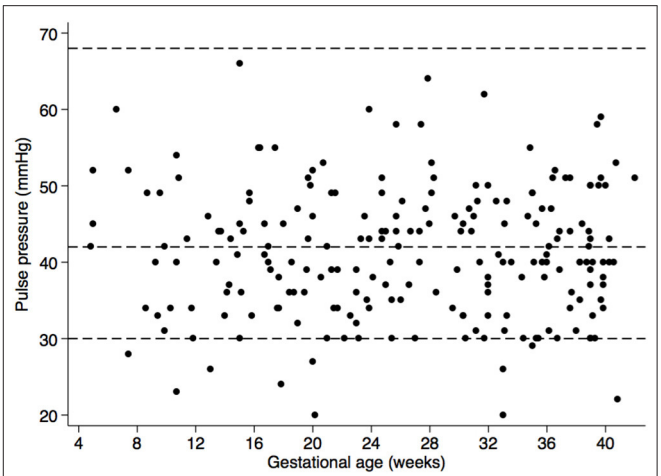

FIGURE 3 | Scatter plot of pulse pressure (PP) according to gestational age. Dashed lines represent the cut-off for high PP (>68 mm Hg) and low PP (<42 mm Hg) based on findings from Ayala and Herminda (43), as well as the 10th centile in our data set (PP <30 mm Hg).

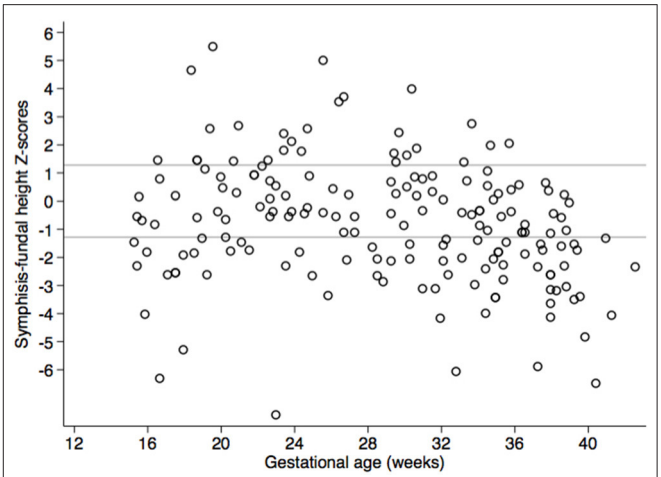

FIGURE 4 | Scatter plot of symphysis-fundal height Z-scores based on INTERGROWTH standards for gestational age in pregnant Ngäbe-Buglé women from rural Panama. Reference lines mark the 10th centile, below which fetuses are considered to be small for gestational age, and the 90th centile, above which fetuses are considered large for gestational age (29).

Blood Pressure and SFH Z-Scores

T-test comparisons of SFH Z-scores based on GA-specific cut-offs for eMAP, MAP<70 mmHg, SBP/DBP < 100/60, and PP < 42 mmHg showed no significant differences. In order to determine whether lowest and highest centiles of the blood pressure measurements (SBP, DBP, MAP, PP) were associated with SFH, each BP measurement was included as an independent factor variable in separate models for SFH Z scores. Only PP emerged as significantly associated with SFH Z-score (**Table 5**). Compared to women whose PP was between the 10 and 90th centile, SFH-Z score was lower in women in the <10th centile but similar to those in the >90th centile (**Figure 5**).

DISCUSSION

The Ngäbe-Buglé indigenous community has one of the highest rates of adverse pregnancy outcomes in Panama, due in part to its remoteness and difficult access to health care, making it imperative from a public health perspective, to be better able to detect women at high risk. Compared with traditional reliance on SBP and DBP, we have observed that MAP and PP provided important information about the risk of both high and low blood pressure in our study population. High MAP has been validated as a risk factor for HDPs (58), and in fact, eMAP was the only BP measurement that allowed us to identify risk of HDPs in our MINDI cohort (11.3%).

TABLE 2 | Multiple stepwise linear regression models for systolic blood pressure (SBP) and diastolic blood pressure (DBP) in pregnant Ngäbe-Buglé women from rural Panama.

A. SBP[a,c]	**Coefficient ± SE**	***P***	**95% CI**	**β**	**Overall model**
GA, wks	0.09 ± 0.08	0.279	−0.07, 0.26	0.091	$P < 0.0001$
Weight for height classification	4.08 ± 1.44	0.005	1.23, 6.93	0.239	Adj. $R^2 = 0.313$
MNS, tbsp/d	1.53 ± 0.50	0.003	0.54, 2.52	0.262	
TNF-α, pg/mL	0.35 ± 0.09	<0.0001	0.16, 0.54	0.294	
Caries, presence	−3.3 ± 1.89	0.075	−7.14, 0.35	−0.142	
Scabies, presence	−4.28 ± 1.89	0.026	−8.03, −0.53	−0.182	
Trichomonas, presence	−6.99 ± 1.82	<0.0001	−10.62, −3.38	−0.309	
Hookworm, presence	3.21 ± 1.61	0.049	0.02, 6.40	0.162	
Constant	91.20 ± 4.18	<0.0001	82.91, 99.49		
B. DBP [b,c]	**Coefficient ± SE**	***P***	**95% CI**	**β**	**Overall model**
GA, wks	0.12 ± 0.09	0.173	−0.05, 0.29	0.139	$P < 0.0001$
Weight for height classification	2.45 ± 1.30	0.063	−0.13, 5.04	0.166	Adj. $R^2 = 0.219$
Urinary gravity >1,020	3.34 ± 1.72	0.054	−0.06, 6.75	0.164	
MNS, tbsp/d	0.98 ± 0.46	0.036	0.06, 1.91	0.195	
Iron supplement	−3.98 ± 2.08	0.059	−8.11, 0.14	−0.183	
RBP <30 mg/L	4.44 ± 1.79	0.015	1.88, 8.00	0.214	
Folic acid <10 nmol/L	2.85 ± 1.78	0.113	−0.69, 6.38	0.136	
Ascaris, presence	−3.77 ± 1.57	0.018	−6.88, −0.65	−0.207	
Hookworm, presence	3.04 ± 1.50	0.046	0.06, 6.02	0.177	
Constant	51.51 ± 3.58	<0.0001	44.40, 58.62		

[a] n = 116. Missing data for TNF-α (1), Trichomonas (2), hookworm (93). Little's Chi-squared test for randomness of missing data, P = 0.698.
VIF = 1.11. Condition number = 15.29.
Variables that were included but had a P > 0.10: wood smoke exposure (0 = no, 1 = yes), hematocrit quantile, IL-13 (pg/mL), IL-17 (pg/mL), bacteriuria score, Ascaris (0 = no, 1 = yes).
[b] n= 116. Missing data for RPB (1), urinary gravity (5), hookworm (93), Ascaris (93). Little's Chi-squared test for randomness of missing data, P = 0.197.
VIF = 1.18. Condition number = 15.23.
Variables that were included but had a P>0.10: hematocrit quantile, hemoglobin (g/L), IL-13 (pg/mL), vitamin D<50 nmol/L, Trichomonas (0 = no, 1 = yes).
[c] Unstandardized coefficient and standardized β. Binary variables (urinary gravity > 1,020, taking iron supplements and presence of infections) were included as 0 = no, 1 = yes.
GA, gestational age; MNS, multiple micronutrient supplement; RBP, retinol-binding protein.

Furthermore, few studies have investigated the prevalence of low BP in marginalized communities. We identified a high prevalence of low BP, and importantly, low PP was associated with the lowest SFH for gestational age. These findings emphasize importance of routine blood pressure measurements in remote areas to identify women at risk of poor pregnancy outcomes. Consistent with our hypothesis, we also provide evidence that individual infections, nutrient deficiencies and cytokines, our markers of inflammation, differentially modified each blood pressure measurement, and may account for the normal values for SPB and DPB, despite evidence of high and low blood pressure based on MAP and PP. With regard to the presence of multiple infections, those that were associated with an anti-inflammatory (Th2) response (*Ascaris, Trichomonas,* scabies) were associated with lower blood pressure (SBP, DBP, MAP, and SBP/DBP <100/60 mmHg) whereas those associated with a pro-inflammatory (Th1) response (hookworm, *Trichuris*, UTI) were associated with higher blood pressure measurements (SBP, DBP, and MAP). With regard to inflammation, we found that TNF-α, which is a hallmark cytokine for HDPs, was associated not only with higher SBP but also with higher MAP, supporting the use of MAP for early detection of women at risk of HDPs. In contrast, IL-17, known to increase with placental hypoperfusion, was negatively associated with PP. With regards to specific nutritional deficiencies, low protein and folic acid deficiency were associated with higher MAP and eMAP, respectively. Thus against the backdrop of low blood pressure in nearly 25% of the population, we report for the first time that higher intakes of MNS and animal source foods were associated with reduced likelihood of low blood pressure.

Nutrition and Blood Pressure

There is growing evidence that blood pressure is modulated by several nutrients and not just sodium (59). Recently the INTERMAP Study (60) identified protein, insoluble fiber, phosphorus, calcium, magnesium, and non-heme iron as having inverse relationships with SBP and DBP. In our study, pregnant mothers consumed a poor diet leading to protein and micronutrient deficiencies, but were provided with a dietary supplement by the Ministry of Health (61) containing energy (400 kcal), protein (12.0 g), lipids (12–14 g), and several micronutrients (vitamins A, E, B_1, B_2, B_3, B_6, B_{12}, folic acid, calcium, phosphorus, iron, zinc, and copper). Noteworthy among our findings was the observation that even though women in

TABLE 3 | Multiple stepwise linear regression models for mean arterial pressure (MAP), and pulse pressure (PP) in pregnant Ngäbe-Buglé women from rural Panama.

A. MAP[a, c]	**Coefficient ± SE**	***P***	**95% CI**	**β**	**Overall model**
GA, wks	0.07 ± 0.07	0.293	−0.06, 0.21	0.092	$P < 0.0001$
Weight for height classification	3.24 ± 1.17	0.007	0.91, 5.56	0.233	Adj. $R^2 = 0.294$
Urinary gravity > 1,020	3.03 ± 1.55	0.053	−0.03, 6.10	0.158	
MNS, tbsp/d	1.00 ± 0.42	0.020	0.16, 1.84	0.210	
TNF-α, pg/mL	0.17 ± 0.08	0.033	0.01, 0.33	0.178	
Trichomonas, presence	−4.02 ± 1.52	0.009	−7.03, −1.00	−0.217	
RBP < 30 mg/L	4.00 ± 1.61	0.015	0.81, 7.19	0.205	
Ascaris, presence	−3.13 ± 1.40	0.028	−5.91,−0.35	−0.182	
Hookworm, presence	3.28 ± 1.34	0.016	0.63, 5.94	0.204	
Constant	63.6 ± 3.4	<0.0001	56.9, 70.3		
B. PP[b,c]	**Coefficient ± SE**	***P***	**95% CI**	**β**	**Overall model**
GA, wks	−0.05 ± 0.06	0.355	−0.18, 0.06	−0.066	$P = 0.0007$
Age, yrs	−0.20 ± 0.08	0.013	−0.36, −0.04	−0.169	Adj. $R^2 = 0.083$
MNS, tbsp/d	0.79 ± 0.38	0.040	0.03, 1.55	0.145	
Basophils, number/mm^3	0.06 ± 0.04	0.110	−0.01, 0.14	0.109	
IL-17, pg/mL	−0.24 ± 0.07	0.002	−0.39, −0.09	−0.290	
TNF-α, pg/mL	0.27 ± 0.09	0.005	0.08, 0.46	0.261	
Constant	44.58 ± 2.83	<0.0001	39.0, 50.16		

[a] n= 116. Missing data for TNF-α (1), RBP (1), Trichomonas (2), urinary gravity (5), hookworm (93), Ascaris (93). Little's Chi-squared test for randomness of missing data, P = 0.163. VIF = 1.14. Condition number = 15.63. Variables that were included but had a P > 0.10: folic acid <10 nmol/L, hematocrit quantile, urinary protein score, hemoglobin (g/L), IL-13 (pg/mL), vitamin D <50 nmol/L, vaginal yeast score.

[b] n = 206. Missing data for TNF-α (1), IL-17 (1), Mobiluncus and Bacteroides/Gardnerella (2), urinary gravity (5). Little's Chi-squared test for randomness of missing data, P = 0.928. VIF = 1.36. Condition number = 13.31.

Variables that were included but had a P > 0.10: neutrophils (#), urinary gravity, Mobiluncus and Bacteroides/Gardnerella scores.

[c] MAP = DBP + 0.33 [SBP-DBP]; PP = SBP-DBP (3). Unstandardized coefficient and standardized β. Binary variables (urinary gravity > 1,020 and presence of infections) were included as 0 = no, 1 = yes.

GA, gestational age; BMI, body mass index; MNS, multiple micronutrient supplement; RBP, retinol binding protein.

our study consumed less of the MNS than recommended, its intake was associated with a slightly higher SBP, DBP, MAP, and PP, which aligns with a previous MNS study showing that folic acid supplementation was also positively associated with SBP, DBP, MAP, and PP in a high-income setting (62). In our study, the MNS was also associated with decreasing the odds of BP < 100/60, suggesting that adequate intake of one or more of the nutrients included in the MNS might help normalize the low blood pressure in our population of pregnant women.

One characteristic of our population is the intake of diluted coffee. It is known that caffeine at dosages from by 80 to 300 mg can increase SBP by about 3–8 mmHg and DBP by about 4–6 mmHg (63). In our population, coffee concentrations had a median of 2.6 (range 6.5–24.5) mg/100 mL, and coffee intake was associated not with higher but with lower MAP, and only in the first trimester. A beneficial effect of polyphenols contained in coffee (64, 65) or a higher intake of fluids with positive effect on cardiovascular health (66) may account for this association.

Several studies have examined the consequences of maternal dietary protein intake on pregnancy outcomes, particularly SGA. Although a Cochrane review concluded that there is no justification for prescribing high-protein nutritional supplements to pregnant women (67), a more recent meta-analysis of studies from low-medium income countries found that balanced protein-energy supplementation of undernourished women significantly improved birthweight (68). Although the link between maternal protein malnutrition and hypertension in the offspring has been established (69), one study with Dutch women showed that protein intake-related acid load was not associated with HDPs, in protein-replete women (70). However, a higher vegetable protein/potassium ratio was associated with lower DBP (70), which is consistent with a meta-analysis in non-pregnant populations from developed countries that showed that increased intake of dietary protein relative to carbohydrate was associated with lower blood pressure (71). In our marginalized community, there was clinical evidence of dietary protein deficiency with 26% of mothers having low RBP and 85% having low B_{12}, but our finding that higher intakes of animal source foods reduced the odds of low blood pressure agrees with observations from developing settings where higher protein intakes in the context of carbohydrate-rich diets could decrease blood pressure (71, 72). Our study is the first to observe an association of higher protein intake with the reduced odds of hypotension in marginalized communities.

Another nutrient deficiency that emerged as increasing the odds for elevated MAP was folic acid. We had previously shown that folic acid deficiency was positively associated with elevated CRP in lactating women in our population (32), which aligns

TABLE 4 | Multiple logistic regression model for elevated mean arterial pressure (eMAP) and low blood pressure in pregnant Ngäbe-Buglé women from rural Panama.

A. eMAP[a,c]	**Odds Ratio ± SE**	***P***	**95% CI**	**Overall model**
Age, yrs	1.18 ± 0.07	0.003	1.06, 1.32	$P = 0.0003$
Folic acid <10 nmol/L	6.98 ± 5.62	0.016	1.44, 33.79	Pseudo $R^2 = 0.272$
Trichuris, presence[c]	6.72 ± 6.48	0.048	1.01, 44.56	
Constant	0.0003 ± 0.0005	<0.0001	5.6^{-6}, 0.01	
B. Low blood pressure[b,c]	**Odds Ratio ± SE**	***P***	**95% CI**	**Overall model**
GA, wks	0.93 ± 0.03	0.036	0.87, 0.99	$P < 0.0001$
BMI, kg/m^2	0.75 ± 0.07	0.002	0.62, 0.90	Pseudo $R^2 = 0.418$
Field work, h/d	0.60 ± 0.09	0.001	0.45, 0.81	
Ascaris, presence [c]	3.63 ± 2.28	0.040	1.06, 12.41	
MNS, tbsp/d	0.35 ± 0.11	0.001	0.19, 0.65	
IL-17, pg/mL^3	0.87 ± 0.05	0.016	0.78, 0.97	
IFN-γ, pg/mL^3	1.08 ± 0.05	0.082	0.99, 1.18	
Hookworm, presence[c]	0.33 ± 0.21	0.080	0.09, 1.14	
Animal-source foods/wk	0.70 ± 0.10	0.012	0.54, 0.92	
Constant	62975.3 ± 190587	<0.0001	167.1, 2.47	

[a]eMAP defined as >87 mmHg between weeks 10–18, >84 mmHg in weeks 18–34, and >86 mmHg after week 34 (41).
n = 117. Missing data for IFNγ (1), IL-1β (1), IL-17 (1), RBP (1), bacteriuria (5), Ascaris (93), Trichuris (93), hookworm (93). Little's Chi-squared test for randomness of missing data, P = 0.486.
VIF = 1.03. Condition number = 7.76.
Variables that were included but had a P > 0.10: BMI category, NLR, MCV (fL), IL-1β (pg/mL), IL-17 (pg/mL), iron supplementation (mo), MNS supplementation (tbsp/d), RBP < 30 mg/L, Ascaris (0 = no, 1 = yes), vaginal yeast (0 = no, 1 = yes), bacteriuria (0 = no, 1 = yes).
[b]Low blood pressure defined as SBP <100 and DBP <60 mmHg (3).
n = 119. Missing data for IFNγ (1), IL-17 (1), RBP (1), bacteriuria (5), Ascaris (93), Trichuris (93), hookworm (93). Two outliers of IL-17 were removed. Little's Chi-squared test for randomness of missing data, P = 0.579.
VIF = 1.32. Condition number = 27.44.
Variables that were included but had a P > 0.10: hematocrit quantile, green leafy vegetable intake/wk, iron supplementation (mo), folic acid <10 nmol/L, vitamin D (nmol/L), scabies (0 = no, 1 = yes).
[c]Binary variables (folic acid <10 nmol/L and presence of infections) were included as 0 = no, 1 = yes.
GA, gestational age; BMI, body mass index; MNS, multiple micronutrient supplement; RBP, retinol binding protein; NLR, neutrophil-lymphocyte ratio; MCV, mean corpuscular volume.

TABLE 5 | Multiple linear regression models of symphysis fundal height (SFH) Z-scores and pulse pressure (PP) in 177 pregnant Ngäbe-Buglé women from rural Panama with gestational age ≥ 16 wks.

Adjusted Model for SFH-Z score[a,b,c]	**Coefficient ± SE**	***P***	**95% CI**	**β**	**Overall Model**
PP < 10th centile (<30 mmHg)	−2.85 ± 0.77	<0.0001	−4.37, −1.32	−0.262	$P < 0.0001$
PP > 90th centile (>51.6 mmHg)	−0.06 ± 0.53	0.899	−1.12, 0.98	−0.009	Adj. $R^2 = 0.117$
Weight for height classification	0.57 ± 0.26	0.029	0.06, 1.08	0.158	
Wood smoke (h/d)	−0.30 ± 0.09	0.002	−0.50, −0.11	−0.222	
Constant	−2.00 ± 0.60	0.001	−3.19, −0.80		

[a]A three-level factor was used for our independent variable, pulse pressure (< 10th centile, ≥ 10–≤90th centiles, and > 90th centile), and comparisons were made against the base category (≥10–≤90th centile). Adjusted for BMI category and wood smoke exposure (h/d).
[b]n = 177. Missing data for SFH (15). Little's Chi-squared test for randomness of missing data, P = 0.466.
VIF = 1.02. Condition number = 15.45.
[c]SFH, symphysis-fundal height; PP, pulse pressure.

with evidence that folic acid deficiency may promote increased BP probably as consequence of inflammation (73). Folic acid supplementation for the prevention of neural tube defects and low birthweight has been studied (74), but its use for the prevention of HDPs is still controversial. Although folic acid supplementation did not change SBP or DBP in women from Iran (75) or The Netherlands (62), and although folic acid levels were similar between normal and hypertensive pregnancies in India (76), it has been reported that folate supplementation decreased the risk of HDPs in populations from China (77) and Korea (78). The association of folic acid deficiency with increased odds of eMAP points toward a benefit of folic acid supplementation for the prevention of HDPs and aligns with the significant positive correlations of serum homocysteine with high MAP in women with pregnancy complicated by HDP in Pakistan (17).

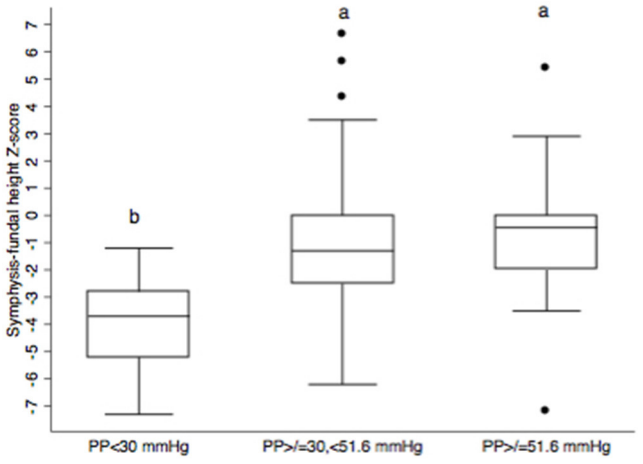

FIGURE 5 | Box and whisker plots of symphysis-fundal height for three pulse pressure (PP) categories (<10th centile, 10–90th centile, and ≥90th centile) based on data from 177 pregnant Ngäbe-Buglé women from rural Panama. The bottom and top of each box represent the 25 and 75th percentiles, respectively; the horizontal line inside the box represents the median. Whiskers show the minimum and maximum values and dots represent outside values. Different lower case letters denote significant differences at $P \leq 0.05$ (a significantly higher than b).

BP and Infections

A particularly intriguing and novel observation was the bi-directional nature of associations between infections and blood pressure indicators. Hookworm was associated with higher SBP, DBP, and MAP and also was associated with lower odds of low blood pressure, and *Trichuris* was associated with increased odds of eMAP. The only data linking this type of infection with blood pressure comes from studies with an intestinal nematode *Strongyloides venezuelensis* that is used as a hookworm model in rodents. This nematode infection was found to increase both SBP and MAP in rats, possibly reflecting the impact of nematode-induced inflammation on blood pressure (79). These results are consistent with the higher blood pressure observed in our pregnant women who were infected with hookworm. In contrast, a third intestinal nematode, *Ascaris*, as well as the scabies mite and the vaginal protozoan, *Trichomonas,* were associated with lower blood pressure and *Ascaris* also increased the odds of low blood pressure.

The contrasting direction of association for *Ascaris,* scabies and trichomoniasis with blood pressure compared with hookworm and *Trichuris* is consistent with the distinctiveness of the inflammatory and immunological responses that they induce. First, in these same pregnant women, CRP was shown to be negatively associated with *Ascaris* but positively associated with hookworm (32), suggesting that *Ascaris* exerted an anti-inflammatory influence whereas hookworm induced a pro-inflammatory response. Second, intestinal nematodes including *Ascaris* (80), scabies mites (81), and *Trichomonas vaginalis* (82, 83) are able to modulate the host immune response with production of classical Th2 cytokines, IL-4, IL-5, and IL-13, mastocytosis, eosinophilia, IgE, and alternatively-activated macrophages known to play a critical role in tissue repair (84). In contrast, hookworm releases molecules that down-regulate the strong Th2 response through a mixed Th2/Th1 response with elevation of pro-inflammatory cytokines including TNF-α (85–87) similar to the pro-inflammatory response observed in response to chronic low dose infection of mice with *Trichuris muris* (88). Taken together, it is not surprising that the association with blood pressure differed for *Ascaris,* scabies, and trichomoniasis compared with hookworm and *Trichuris,* and it is likely that this was linked to the associated anti-inflammatory and pro-inflammatory responses, respectively.

Blood Pressure, Cytokines, and Inflammation

TNF-α was positively associated with SBP, MAP, and PP. Concentrations of TNF-α are known to increase under placental hypoperfusion, and TNF-α is able to increase BP by activating humoral and endothelial factors (89). As TNF-α has been consistently found to be elevated in women with HDPs (90–93), as our TNF-α values were within ranges associated with HDPs, and as we observed a positive association between TNF-α and MAP, our data suggest that eMAP may be a useful early indicator of women at risk for HDPs in MINDI populations.

IL-17 has also been linked to hypertension not only by producing pro-inflammatory and endothelial damage, but also by up-regulating transport channels in the tubules of the kidney (90). This raises an interesting question regarding hemodynamics in these pregnant women, given that hypoproteinemia was common. It is known that in protein malnutrition, there is an increase in renal vascular resistance, renal blood flow and sodium excretion (94). Therefore, the pro-inflammatory response measured through IL-17 might be helping to compensate for hypovolemia by increasing sodium reabsorption and BP, at the expense of perfusion indicated by PP (3). In support of this hypothesis, recent studies have shown that the number of Th17 cells or the concentration of IL-17 is higher in women with hypertension during pregnancy (95, 96), in agreement with our observation that IL-17 was associated with decreased odds of hypotension. Also, experimental studies have shown that Th17 cells from animals with reduced uterine perfusion are able to increase BP and to induce IUGR when injected to normal pregnant animals (97), in agreement with our association of higher IL-17 with decreased PP, and our further observation that low PP was associated with small SFH.

BP Measurements and Symphysis-Fundal Height

Although ultrasound is considered the gold standard for detecting SGA (27), it was not available for our population at the time of the study, and clinicians in the field used SFH as equivalent for estimating fetal growth, based on Pan American Health Organization standards that have reported a sensitivity of 52% and specificity of 92% for the detection of SGA (28). Despite the large sample size of the INTERGROWTH study, authors did

not consider it appropriate to make recommendations about the cutoff values for SGA (29). We overcame this limitation by using the continuous variable of SFH Z scores as our outcome in order to describe its associations with BP measurements.

Hypertension during pregnancy is a known cause of IUGR (98), and although we found women at risk of HDPs based on elevated MAP, none of the blood pressure indicators was associated with low SFH. This differs from several other studies. Higher PP was associated with lower birthweight in a cohort of 50 normotensive women from the Netherlands (99). Also, European (100) and Asian studies (101) have shown that elevated SBP and DBP were associated with impaired fetal growth. Although few studies have addressed hypotension as a potential risk factor for SGA, we found that low PP (but not low SBP, DBP, or MAP) was associated with SGA fetuses in our cohort of women participating in normal pregnancy follow up. It is known that women who do not achieve the physiologic increase of blood volume are prone to HDPs, fetal growth restriction and SGA (102, 103). The risk of stillbirth has been reported to be higher in women with low DBP and low MAP (9), and low BP defined as DBP < 80 mmHg was associated with preterm birth and SGA although this association was lost after controlling for maternal age, BMI, socio-economic status, and race (8). Our findings demonstrate that hypotension may be an unrecognized factor contributing to impaired SFH in our MINDI cohort.

Limitations

Despite the increased need for research to understand major health issues in remote settings, a recent review shows that among clinical research studies, 3.27% are dedicated to women, 1.72% to the poor, 1.66% to rural residents and 1.55% to visible minorities (104). Our study may not be comparable to populations where most clinical research originates, but it reflects the reality of an important proportion of the world population, where women have limited access to technology and where the utility of simple biomarkers such as blood pressure and SFH needs to be maximized.

Data on birth outcomes was not available given that most women delivered at home, a limitation that is shared by clinicians in their daily practice during routine pregnancy follow-ups. Just as field clinicians, we needed to make the best use of available resources to identify pregnant women at risk. Despite this limitation, our comprehensive database allowed us to identify novel associations of BP measurements with MINDI and to provide evidence that MAP and PP, together with SFH, may help in the identification of women who are at high risk to develop complications.

CONCLUSION

Our results demonstrate that routine blood pressure measurements, in particular eMAP, which reflects overall exposure to multiple infections, nutritional deficiencies and inflammation, can be used to assess at-risk pregnancies in remote settings where SBP and DBP were unable to detect risk of HDPs. Our findings also showed that protein deficiency was associated with higher MAP and that folate deficiency was associated with increased odds of eMAP, suggesting that current folic acid and protein supplementation need to be strengthened to meet the needs during pregnancy. Our novel finding of associations of infections with SBP, DBP, and MAP call for the need to consider that *Ascaris*, *Trichomonas* and scabies may lower BP, whereas hookworm, *Trichuris* and UTI may increase BP, and have implications for deworming programs during pregnancy. Furthermore, given that neither SBP, DBP, nor MAP were associated with SFH, but PP was, the association of low PP with smaller SFH makes it a promising indicator for women at risk of adverse pregnancy outcomes in remote areas where sonography is not available.

DATA AVAILABILITY STATEMENT

The datasets for this article are not publicly available because participants did not sign informed consent that data will be publicly available, neither was this possibility discussed with Ethical Boards in Panama or Canada. Requests to access the datasets should be directed to Dr. Kristine G. Koski (kristine.koski@mcgill.ca).

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Gorgas Memorial Institute Research Bioethics Committee (Panama) and McGill University Research Ethics Board. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

DG-F contributed to study design and conceptual framework, data collection and statistical analysis, and writing of the final manuscript. EP, DR, OS, and EM contributed to the study design, coordinated field and laboratory analyses, read, and approved the final manuscript. MS contributed to the study design, conceptual framework, and read and approved the final manuscript. KK contributed to study design and conceptual framework, data collection, and statistical analysis and writing of the final manuscript. EM, MS and KK were involved in funding the study.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2020.00086/full#supplementary-material

REFERENCES

1. Madaj B, Smith H, Mathai M, Roos N, van den Broek N. *Developing Global Indicators for Quality of Maternaland Newborn Care: A Feasibility Assessment*. Geneva: Bulletin of the World Health Organization (2017). Available online at: http://www.who.int/bulletin/volumes/95/6/16-179531/en/
2. Macdonald-Wallis C, Silverwood RJ, Fraser A, Nelson SM, Tilling K, Lawlor DA, et al. Gestational-age-specific reference ranges for blood pressure in pregnancy: findings from a prospective cohort. *J Hypertens.* (2015) 33:96–105. doi: 10.1097/hjh.0000000000000368
3. Klabunde RE. Vascular Function, In: Taylor C, editor. *Cardiovascular Physiology Concepts*. 2nd ed. Philadelphia, PA: Wolters Kluwer Lippincott Williams & Wilkins (2012). p. 97–8.
4. Steyn W. Pp136. blood pressure patterns in pregnant women who developed pre-eclampsia or hypertension. *Pregnancy Hypertens.* (2012) 2:312–4. doi: 10.1016/j.preghy.2012.04.247
5. Lai J, Poon LC, Bakalis S, Chiriac R, Nicolaides KH. Systolic, diastolic and mean arterial pressure at 30-33 weeks in the prediction of preeclampsia. *Fetal Diagn Ther.* (2013) 33:173–81. doi: 10.1159/000345950
6. Adsumelli RS, Elimian A, Wiencek V, Benveniste HD, Glass PS, Quirk JG. Change in pulse pressure during the preclinical phase of preeclampsia. *J Reprod Med.* (2006) 51:26–30. Available online at: http://www.reproductivemedicine.com/toc/auto_abstract.php?id=22370
7. Hale S, Choate M, Schonberg A, Shapiro R, Badger G, Bernstein IM. Pulse pressure and arterial compliance prior to pregnancy and the development of complicated hypertension during pregnancy. *Reprod Sci.* (2010) 17:871–7. doi: 10.1177/1933719110376545
8. Zhang J, Klebanoff MA. Low blood pressure during pregnancy and poor perinatal outcomes: an obstetric paradox. *Am J Epidemiol.* (2001) 153:642–6. doi: 10.1093/aje/153.7.642
9. Warland J, McCutcheon H, Baghurst P. Maternal blood pressure in pregnancy and stillbirth: a case-control study of third-trimester stillbirth. *Am J Perinatol.* (2008) 25:311–7. doi: 10.1055/s-2008-1075031
10. Imdad A, Bhutta ZA. Nutritional management of the low birth weight/preterm infant in community settings: a perspective from the developing world. *J Pediatr.* (2013) 162(Suppl. 3):S107–14. doi: 10.1016/j.jpeds.2012.11.060
11. Bej P, Chhabra P, Sharma AK, Guleria K. Determination of risk factors for pre-eclampsia and eclampsia in a tertiary hospital of India: a case control study. *J Family Med Prim Care.* (2013) 2:371–5. doi: 10.4103/2249-4863.123924
12. Bilano VL, Ota E, Ganchimeg T, Mori R, Souza JP. Risk factors of pre-eclampsia/eclampsia and its adverse outcomes in low- and middle-income countries: a WHO secondary analysis. *PLoS ONE.* (2014) 9:e91198. doi: 10.1371/journal.pone.0091198
13. Gaillard R, Eilers PH, Yassine S, Hofman A, Steegers EA, Jaddoe VW. Risk factors and consequences of maternal anaemia and elevated haemoglobin levels during pregnancy: a population-based prospective cohort study. *Paediatr Perinat Epidemiol.* (2014) 28:213–26. doi: 10.1111/ppe.12112
14. Heilmann L, Siekmann U, Schmid-Schonbein H, Ludwig H. Hemoconcentration and pre-eclampsia. *Arch Gynecol.* (1981) 231:7–21. doi: 10.1007/BF02110019
15. Barebring L, Bullarbo M, Glantz A, Leu Agelii M, Jagner A, Ellis J, et al. Preeclampsia and blood pressure trajectory during pregnancy in relation to vitamin D status. *PLoS ONE.* (2016) 11:e0152198. doi: 10.1371/journal.pone.0152198
16. Gaiday AN, Tussupkaliyev AB, Bermagambetova SK, Zhumagulova SS, Sarsembayeva LK, Dossimbetova MB, et al. Effect of homocysteine on pregnancy: a systematic review. *Chem Biol Interact.* (2018) 293:70–6. doi: 10.1016/j.cbi.2018.07.021
17. Bibi S, Qureshi, Ahmad M, Qureshi PM, Memon A, Qazi RA. Hyperhomocysteinaemia, vascular related pregnancy complications and the response to vitamin supplementation in pregnant women of Pakistan. *J Pak Med Assoc.* (2010) 60:741–5. Available online at: https://jpma.org.pk/article-details/2293?article_id=2293
18. Zhang C, Williams MA, Sanchez SE, King IB, Ware-Jauregui S, Larrabure G, et al. Plasma concentrations of carotenoids, retinol, and tocopherols in preeclamptic and normotensive pregnant women. *Am J Epidemiol.* (2001) 153:572–80. doi: 10.1093/aje/153.6.572
19. Williams MA, Woelk GB, King IB, Jenkins L, Mahomed K. plasma carotenoids, retinol, tocopherols, and lipoproteins in preeclamptic and normotensive pregnant Zimbabwean women. *Am J Hypertens.* (2003) 16:665–72. doi: 10.1016/S0895-7061(03)00897-5
20. Rulisa S, Kaligirwa N, Agaba S, Karangayire P, Mens PF, de Vries PJ. Fetal and maternal hemodynamics in acute malaria during pregnancy. *Int J Gynaecol Obstet.* (2012) 119:66–9. doi: 10.1016/j.ijgo.2012.04.024
21. Alshareef SA, Nasr AM, Adam I. *Toxoplasma Gondii* infection and pre-eclampsia among Sudanese women. *Trans R Soc Trop Med Hyg.* (2018) 112:393–6. doi: 10.1093/trstmh/try067
22. Lopez-Lopez J, Lopez-Jaramillo P, Camacho PA, Gomez-Arbelaez D, Cohen DD. The link between fetal programming, inflammation, muscular strength, and blood pressure. *Mediators Inflamm.* (2015) 2015:710613. doi: 10.1155/2015/710613
23. Paternoster DM, Fantinato S, Stella A, Nanhorngue KN, Milani M, Plebani M, et al. C-reactive protein in hypertensive disorders in pregnancy. *Clin Appl Thromb Hemost.* (2006) 12:330–7. doi: 10.1177/1076029606291382
24. Onuegbu AJ, Olisekodiaka JM, Udo JU, Umeononihu O, Amah UK, Okwara JE, et al. Evaluation of high-sensitivity C-reactive protein and serum lipid profile in Southeastern Nigerian women with pre-eclampsia. *Med Princ Pract.* (2015) 24:276–9. doi: 10.1159/000381778
25. Udenze I, Amadi C, Awolola N, Makwe CC. The role of cytokines as inflammatory mediators in preeclampsia. *Pan Afr Med J.* (2015) 20:219. doi: 10.11604/pamj.2015.20.219.5317
26. Harmon AC, Cornelius DC, Amaral LM, Faulkner JL, Cunningham MW Jr, et al. The role of inflammation in the pathology of preeclampsia. *Clin Sci.* (2016) 130:409–19. doi: 10.1042/CS20150702
27. WHO Reproductive Health Library. *WHO Recommendation on Symphysis-Fundal Height Measurement*. Geneva: World Health Organization (2018). Available online at: https://extranet.who.int/rhl/topics/preconception-pregnancy-childbirth-and-postpartum-care/antenatal-care/who-recommendation-symphysis-fundal-height-measurement (accessed March 12, 2020).
28. Fescina R H, De Mucio B, Martínez G, Alemán A, Sosa C, Mainero L, et al. *Monitoring Fetal Growth: Self-Instruction Manual*: Pan American Health Organization, Latin American Center for Perinatology/Women & Reproductive Health (2011) Available online at: http://www.paho.org/Clap/index.php?option=com__docman&task=doc__details&gid=241&tmpl=component&Itemid= (accessed January 7, 2017).
29. Papageorghiou AT, Ohuma EO, Gravett MG, Hirst J, da Silveira MF, Lambert A, et al. International standards for symphysis-fundal height based on serial measurements from the fetal growth longitudinal study of the Intergrowth-21st project: prospective cohort study in eight countries. *BMJ.* (2016) 355:i5662. doi: 10.1136/bmj.i5662

30. PNUD Panamá. *Objetivos de Desarrollo del Milenio, Tercer Informe de Panamá, 2009 [Millennium Development Goals, Third Report of Panama, 2009]*. Panama: United Nations Development Programme (2009). Available online at: http://www.pa.undp.org/content/panama/es/home/library/mdg/odm-tercer-informe-de-panama-2009.html (accessed December 12, 2017).
31. Kuc S, Koster MP, Franx A, Schielen PC, Visser GH. Maternal characteristics, mean arterial pressure and serum markers in early prediction of preeclampsia. *PLoS ONE.* (2013) 8:e63546. doi: 10.1371/journal.pone.0063546
32. Gonzalez-Fernandez D, Pons EDC, Rueda D, Sinisterra OT, Murillo E, Scott ME, et al. C-reactive protein is differentially modulated by co-existing infections, vitamin deficiencies and maternal factors in pregnant and lactating indigenous Panamanian women. *Infect Dis Poverty.* (2017) 6:94. doi: 10.1186/s40249-017-0307-1
33. INEC. *[National Institute of Statistics and Census] Nacimientos Vivos y Defunciones Fetales: Año 2010 [Live Births and Fetal Deaths: Year 2010]*. Panama: Instituto Nacional de Estadística y Censo (2010). Available online at: https://www.inec.gob.pa/publicaciones/Default3.aspx?ID_PUBLICACION=300&ID_CATEGORIA=3&ID_SUBCATEGORIA=6 (accessed June 11, 2017).
34. Panamanian Ministry of Health. *[Health Situation of Panama 2013]*. Panama: National Directory of Planning (2013). Available online at: http://www.minsa.gob.pa/sites/default/files/publicaciones/situacion_de_salud_panama_2013_0.pdf (accessed December 15, 2017).
35. Gonzalez-Fernandez D, Koski KG, Sinisterra OT, Del Carmen Pons E, Murillo E, Scott ME. Interactions among urogenital, intestinal, skin, and oral infections in pregnant and lactating Panamanian Ngabe women: a Neglected public health challenge. *Am J Trop Med Hyg.* (2015) 92:1100–10. doi: 10.4269/ajtmh.14-0547
36. Vigil-De Gracia P, Rojas-Suarez J, Ramos E, Reyes O, Collantes J, Quintero A, et al. Incidence of eclampsia with hellp syndrome and associated mortality in Latin America. *Int J Gynaecol Obstet.* (2015) 129:219–22. doi: 10.1016/j.ijgo.2014.11.024
37. Abdelaziz A, Maher MA, Sayyed TM, Bazeed MF, Mohamed NS. Early pregnancy screening for hypertensive disorders in women without a-priori high risk. *Ultrasound Obstet Gynecol.* (2012) 40:398–405. doi: 10.1002/uog.11205
38. Bello NA, Woolley JJ, Cleary KL, Falzon L, Alpert BS, Oparil S, et al. Accuracy of blood pressure measurement devices in pregnancy: a systematic review of validation studies. *Hypertension.* (2018) 71:326–35. doi: 10.1161/HYPERTENSIONAHA.117.10295
39. Pickering TG, Hall JE, Appel LJ, Falkner BE, Graves J, Hill MN, et al. Recommendations for blood pressure measurement in humans and experimental animals: part 1: blood pressure measurement in humans: a statement for professionals from the subcommittee of professional and public education of the American heart association council on high blood pressure research. *Circulation.* (2005) 111:697–716. doi: 10.1161/01.CIR.0000154900.76284.F6
40. Magee LA, Pels A, Helewa M, Rey E, von Dadelszen P. Diagnosis, evaluation, and management of the hypertensive disorders of pregnancy: executive summary. *J Obstet Gynaecol Can.* (2014) 36:575–6. doi: 10.1016/S1701-2163(15)30588-0
41. WHEC. *Normal Values in Pregnancy*. Springfield, MA: The Women's Health and Education Organization, Inc. (2009). Available online at: http://www.womenshealthsection.com/content/obs/obs025.php3 (accessed October 11, 2019).
42. Henry JB, Miller MC, Kelly KC, Champney D. Mean arterial pressure (map): an alternative and preferable measurement to systolic blood pressure (SBP) in patients for hypotension detection during hemapheresis. *J Clin Apheresis.* (2002) 17:55–64. doi: 10.1002/jca.10022
43. Ayala DE, Hermida RC. Ambulatory blood pressure monitoring for the early identification of hypertension in pregnancy. *Chronobiol Int.* (2013) 30:233–59. doi: 10.3109/07420528.2012.714687
44. Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. *J Clin Microbiol.* (1991) 29:297–301. doi: 10.1128/JCM.29.2.297-301.1991
45. WHO, CDC. *Assessing the Iron Status of Populations*. Health Topics: Nutrition: WHO, CDC (2007). Available online at: http://apps.who.int/iris/bitstream/10665/75368/1/9789241596107_eng.pdf?ua$=$1&ua=1 (accessed October 3, 2019).
46. Abbassi-Ghanavati M, Greer LG, Cunningham FG. Pregnancy and laboratory studies: a reference table for clinicians. *Obstet Gynecol.* (2009) 114:1326–31. doi: 10.1097/AOG.0b013e3181c2bde8
47. Coad S, Friedman B, Geoffrion R. Understanding urinalysis: clues for the obstetrician–gynecologist. *Exp Rev Obstetr Gynecol.* (2012) 7:269–79. doi: 10.1586/eog.12.21
48. Gundersen TE, Lundanes E, Blomhoff R. Quantitative high-performance liquid chromatographic determination of retinoids in human serum using on-line solid-phase extraction and column switching. determination of 9-cis-retinoic acid, 13-cis-retinoic acid, all-trans-retinoic acid, 4-oxo-all-trans-retinoicacid and 4-oxo-13-cis-retinoic acid. *J Chromatogr B Biomed Appl.* (1997) 691:43–58. doi: 10.1016/S0378-4347(96)00434-3
49. de Benoist B. Conclusions of a WHO technical consultation on folate and vitamin B_{12} deficiencies. *Food Nutr Bull.* (2008) 29(Suppl. 2):S238–44. doi: 10.1177/15648265080292S129
50. Balvers MG, Brouwer-Brolsma EM, Endenburg S, de Groot LC, Kok FJ, Gunnewiek JK. Recommended intakes of vitamin D to optimize health, associated circulating 25-hydroxyvitamin D concentrations, and dosing regimens to treat deficiency: workshop report and overview of current literature. *J Nutr Sci.* (2015) 4:e23. doi: 10.1017/jns.2015.10
51. Wiseman EM, Bar-El Dadon S, Reifen R. The vicious cycle of vitamin a deficiency: a review. *Crit Rev Food Sci Nutr.* (2016) 57:3703–14. doi: 10.1080/10408398.2016.1160362
52. McCauley ME, van den Broek N, Dou L, Othman M. Vitamin A supplementation during pregnancy for maternal and newborn outcomes. *Cochrane Database Syst Rev.* (2015) 10:CD008666. doi: 10.1002/14651858.CD008666.pub3
53. Sergi G, Coin A, Enzi G, Volpato S, Inelmen EM, Buttarello M, et al. Role of visceral proteins in detecting malnutrition in the elderly. *Eur J Clin Nutr.* (2006) 60:203–9. doi: 10.1038/sj.ejcn.1602289
54. Ashoor SH, Seperich GJ, Monte WC, Welty J. High performance liquid chromatographic determination of caffeine in decaffeinated coffee, tea, and beverage products. *J Assoc Off Anal Chem.* (1983) 66:606–9. doi: 10.1093/jaoac/66.3.606
55. Pigott TD. A review of methods for missing data. *Educ Res Eval.* (2001) 7:353–83. doi: 10.1076/edre.7.4.353.8937
56. Little RJA. A test of missing completely at random for multivariate data with missing values. *JASA.* (1988) 83:1198–202. doi: 10.1080/01621459.1988.10478722
57. Green SB. How many subjects does it take to do a regression analysis. *Multivariate Behav Res.* (1991) 26:499–510. doi: 10.1207/s15327906mbr2603_7
58. Cnossen JS, Vollebregt KC, de Vrieze N, ter Riet G, Mol BW, Franx A, et al. Accuracy of mean arterial pressure and blood pressure measurements in predicting pre-eclampsia: systematic review and meta-analysis. *BMJ.* (2008) 336:1117–20. doi: 10.1136/bmj.39540.522049.BE
59. Kesteloot H, Tzoulaki I, Brown IJ, Chan Q, Wijeyesekera A, Ueshima H, et al. Relation of urinary calcium and magnesium excretion to blood pressure: the international study of macro- and micro-nutrients and blood pressure and the international cooperative study on salt, other factors, and blood pressure. *Am J Epidemiol.* (2011) 174:44–51. doi: 10.1093/aje/kwr049
60. Chan Q, Stamler J, Griep LM, Daviglus ML, Horn LV, Elliott P. An update on nutrients and blood pressure. *J Atheroscler Thromb.* (2016) 23:276–89. doi: 10.5551/jat.30000
61. Panamanian Ministry of Health. *[National Plan for the "Prevention and Control of Micronutrient Deficiencies" 2008-2015]*. Micronutrients National Group (2008). Available online at: https://extranet.who.int/nutrition/gina/sites/default/files/PAN%20Plan%20Nac%20MN%202008-2015.pdf (accessed March 12, 2019).
62. Timmermans S, Jaddoe VW, Silva LM, Hofman A, Raat H, Steegers-Theunissen RP, et al. Folic acid is positively associated with uteroplacental vascular resistance: the generation R Study. *Nutr Metab Cardiovasc Dis.* (2011) 21:54–61. doi: 10.1016/j.numecd.2009.07.002
63. De Giuseppe R, Di Napoli I, Granata F, Mottolese A, Cena H. Caffeine and blood pressure: a critical review perspective. *Nutr Res Rev.* (2019) 32:169–75. doi: 10.1017/S0954422419000015

64. O'Keefe JH, Bhatti SK, Patil HR, DiNicolantonio JJ, Lucan SC, Lavie CJ. Effects of habitual coffee consumption on cardiometabolic disease, cardiovascular health, and all-cause mortality. *J Am Coll Cardiol.* (2013) 62:1043–51. doi: 10.1016/j.jacc.2013.06.035
65. Cherniack EP. Polyphenols: planting the seeds of treatment for the metabolic syndrome. *Nutrition.* (2011) 27:617–23. doi: 10.1016/j.nut.2010.10.013
66. Zhang N, Zhang F, Chen S, Han F, Lin G, Zhai Y, et al. Associations between hydration state and pregnancy complications, maternal-infant outcomes: protocol of a prospective observational cohort study. *BMC Pregnancy Childbirth.* (2020) 20:82. doi: 10.1186/s12884-020-2765-x
67. Kramer MS, Kakuma R. Energy and protein intake in pregnancy. *Cochrane Database Syst Rev.* (2003) 4:Cd000032. doi: 10.1002/14651858.CD000032
68. Stevens B, Buettner P, Watt K, Clough A, Brimblecombe J, Judd J. The effect of balanced protein energy supplementation in undernourished pregnant women and child physical growth in low- and middle-income countries: a systematic review and meta-analysis. *Matern Child Nutr.* (2015) 11:415–32. doi: 10.1111/mcn.12183
69. de Brito Alves JL, Costa-Silva JH. Maternal protein malnutrition induced-hypertension: new evidence about the autonomic and respiratory dysfunctions and epigenetic mechanisms. *Clin Exp Pharmacol Physiol.* (2018) 45:422–9. doi: 10.1111/1440-1681.12892
70. Tielemans MJ, Erler NS, Franco OH, Jaddoe VWV, Steegers EAP, Kiefte-de Jong JC. Dietary acid load and blood pressure development in pregnancy: the generation R Study. *Clin Nutr.* (2018) 37:597–603. doi: 10.1016/j.clnu.2017.01.013
71. Tielemans SM, Altorf-van der Kuil W, Engberink MF, Brink EJ, van Baak MA, Bakker SJ, et al. Intake of total protein, plant protein and animal protein in relation to blood pressure: a meta-analysis of observational and intervention studies. *J Hum Hypertens.* (2013) 27:564–71. doi: 10.1038/jhh.2013.16
72. Teunissen-Beekman KF, van Baak MA. The role of dietary protein in blood pressure regulation. *Curr Opin Lipidol.* (2013) 24:65–70. doi: 10.1097/MOL.0b013e32835b4645
73. Das UN. Hypertension as a low-grade systemic inflammatory condition that has its origins in the perinatal period. *J Assoc Physicians India.* (2006) 54:133–42. Available online at: https://pdfs.semanticscholar.org/40c3/bef48f778217a6a06330cbdcb11e7482e508.pdf
74. Hodgetts VA, Morris RK, Francis A, Gardosi J, Ismail KM. Effectiveness of folic acid supplementation in pregnancy on reducing the risk of small-for-gestational age neonates: a population study, systematic review and meta-analysis. *BJOG.* (2015) 122:478–90. doi: 10.1111/1471-0528.13202
75. Sayyah-Melli M, Ghorbanihaghjo A, Alizadeh M, Kazemi-Shishvan M, Ghojazadeh M, Bidadi S. The effect of high dose folic acid throughout pregnancy on homocysteine (Hcy) concentration and pre-eclampsia: a randomized clinical trial. *PLoS ONE.* (2016) 11:e0154400. doi: 10.1371/journal.pone.0154400
76. Wadhwani NS, Patil VV, Mehendale SS, Wagh GN, Gupte SA, Joshi SR. Increased homocysteine levels exist in women with preeclampsia from early pregnancy. *J Matern Fetal Neonatal Med.* (2016) 29:2719–25. doi: 10.3109/14767058.2015.1102880
77. Wang Y, Zhao N, Qiu J, He X, Zhou M, Cui H, et al. Folic acid supplementation and dietary folate intake, and risk of preeclampsia. *Eur J Clin Nutr.* (2015) 69:1145–50. doi: 10.1038/ejcn.2014.295
78. Kim MW, Ahn KH, Ryu KJ, Hong SC, Lee JS, Nava-Ocampo AA, et al. Preventive effects of folic acid supplementation on adverse maternal and fetal outcomes. *PLoS ONE.* (2014) 9:e97273. doi: 10.1371/journal.pone.0097273
79. Sousa-Ribeiro C, Tavore LNC, Anjos-Ramos L. *Strongyloides venezuelensis* infection augments arterial blood pressure in male wistar rats. *Acta Trop.* (2019) 190:350–5. doi: 10.1016/j.actatropica.2018.12.007
80. Allen JE, Maizels RM. Diversity and dialogue in immunity to helminths. *Nat Rev Immunol.* (2011) 11:375–88. doi: 10.1038/nri2992
81. Bhat SA, Mounsey KE, Liu X, Walton SF. Host immune responses to the itch mite, *Sarcoptes scabiei*, in humans. *Parasit Vectors.* (2017) 10:385. doi: 10.1186/s13071-017-2320-4
82. Mercer F, Johnson PJ. *Trichomonas vaginalis*: pathogenesis, symbiont interactions, and host cell immune responses. *Trends Parasitol.* (2018) 34:683–93. doi: 10.1016/j.pt.2018.05.006
83. Nemati M, Malla N, Yadav M, Khorramdelazad H, Jafarzadeh A. Humoral and T cell-mediated immune response against trichomoniasis. *Parasite Immunol.* (2018) 40:e12510. doi: 10.1111/pim.12510
84. King IL, Li Y. Host-parasite interactions promote disease tolerance to intestinal helminth infection. *Front Immunol.* (2018) 9:2128. doi: 10.3389/fimmu.2018.02128
85. Geiger SM, Caldas IR, Mc Glone BE, Campi-Azevedo AC, De Oliveira LM, Brooker S, et al. Stage-specific immune responses in human *Necator americanus* infection. *Parasite Immunol.* (2007) 29:347–58. doi: 10.1111/j.1365-3024.2007.00950.x
86. Periago MV, Bethony JM. Hookworm virulence factors: making the most of the host. *Microbes Infect.* (2012) 14:1451–64. doi: 10.1016/j.micinf.2012.09.002
87. Fitzsimmons CM, Falcone FH, Dunne DW. Helminth allergens, parasite-specific ige, and its protective role in human immunity. *Front Immunol.* (2014) 5:61. doi: 10.3389/fimmu.2014.00061
88. Holm JB, Sorobetea D, Kiilerich P, Ramayo-Caldas Y, Estelle J, Ma T, et al. Chronic *Trichuris muris* infection decreases diversity of the intestinal microbiota and concomitantly increases the abundance of *Lactobacilli*. *PLoS ONE.* (2015) 10:e0125495. doi: 10.1371/journal.pone.0125495
89. LaMarca BD, Ryan MJ, Gilbert JS, Murphy SR, Granger JP. Inflammatory cytokines in the pathophysiology of hypertension during preeclampsia. *Curr Hypertens Rep.* (2007) 9:480–5. doi: 10.1007/s11906-007-0088-1
90. Wen Y, Crowley SD. Renal effects of cytokines in hypertension. *Adv Exp Med Biol.* (2019) 1165:443–54. doi: 10.1007/978-981-13-8871-2_21
91. Black KD, Horowitz JA. Inflammatory markers and preeclampsia: a systematic review. *Nurs Res.* (2018) 67:242–51. doi: 10.1097/NNR.0000000000000285
92. Szarka A, Rigo J Jr, Lazar L, Beko G, Molvarec A. Circulating cytokines, chemokines and adhesion molecules in normal pregnancy and preeclampsia determined by multiplex suspension array. *BMC Immunol.* (2010) 11:59. doi: 10.1186/1471-2172-11-59
93. Vitoratos N, Economou E, Iavazzo C, Panoulis K, Creatsas G. Maternal serum levels of TNF-alpha and IL-6 long after delivery in preeclamptic and normotensive pregnant women. *Mediators Inflamm.* (2010) 2010:908649. doi: 10.1155/2010/908649
94. Manning RD Jr, Guyton AC. Effects of hypoproteinemia on fluid volumes and arterial pressure. *Am J Physiol.* (1983) 245:H284–93. doi: 10.1152/ajpheart.1983.245.2.H284
95. Zhang Z, Liu H, Shi Y, Xu N, Wang Y, Li A, et al. Increased circulating Th22 cells correlated with Th17 cells in patients with severe preeclampsia. *Hypertens Pregnancy.* (2017) 36:100–7. doi: 10.1080/10641955.2016.1239737
96. Salazar Garcia MD, Mobley Y, Henson J, Davies M, Skariah A, Dambaeva S, et al. Early pregnancy immune biomarkers in peripheral blood may predict preeclampsia. *J Reprod Immunol.* (2018) 125:25–31. doi: 10.1016/j.jri.2017.10.048
97. Cornelius DC, Amaral LM, Wallace K, Campbell N, Thomas AJ, Scott J, et al. Reduced uterine perfusion pressure T-helper 17 cells cause pathophysiology associated with preeclampsia during pregnancy. *Am J Physiol Regul Integr Comp Physiol.* (2016) 311:R1192–r9. doi: 10.1152/ajpregu.00117.2016
98. Nawathe A, David AL. Prophylaxis and treatment of foetal growth restriction. *Best Pract Res Clin Obstet Gynaecol.* (2018) 49:66–78. doi: 10.1016/j.bpobgyn.2018.02.007
99. Elvan-Taspinar A, Franx A, Bots ML, Koomans HA, Bruinse HW. Arterial stiffness and fetal growth in normotensive pregnancy. *Am J Hypertens.* (2005) 18:337–41. doi: 10.1016/j.amjhyper.2004.10.020
100. Bakker R, Steegers EA, Hofman A, Jaddoe VW. Blood pressure in different gestational trimesters, fetal growth, and the risk of adverse birth outcomes: the generation R study. *Am J Epidemiol.* (2011) 174:797–806. doi: 10.1093/aje/kwr151
101. Lim WY, Lee YS, Tan CS, Kwek K, Chong YS, Gluckman PD, et al. The association between maternal blood pressures and offspring size at birth in Southeast Asian women. *BMC Pregnancy Childbirth.* (2014) 14:403. doi: 10.1186/s12884-014-0403-1
102. Hytten F. Blood volume changes in normal pregnancy. *Clin Haematol.* (1985) 14:601–12.

103. de Haas S, Ghossein-Doha C, van Kuijk SM, van Drongelen J, Spaanderman ME. Physiological adaptation of maternal plasma volume during pregnancy: a systematic review and meta-analysis. *Ultrasound Obstet Gynecol.* (2017) 49:177–87. doi: 10.1002/uog.17360
104. Rolnitsky A, Kirtsman M, Goldberg HR, Dunn M, Bell CM. The representation of vulnerable populations in quality improvement studies. *Int J Qual Health Care.* (2018) 30:244–9. doi: 10.1093/intqhc/mzy016
105. González-Fernández D. *Interpretation of maternal and fetal biomarkers in a population with coexisting nutrient deficiencies and infections* (Ph.D. thesis). Montreal, QC: McGill University (2019).
106. González-Fernández D, Pons EDC, Rueda D, Sinisterra OT, Murillo E, Scott ME, et al. Diet, nutritional indicators, infections and inflammation are associated with mean arterial pressure (MAP) in indigenous pregnant and lactating women in rural Panama. *FASEB J.* (2017) 31:147.8. Available online at: https://www.fasebj.org/doi/abs/10.1096/fasebj.31.1_supplement.147.8

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Pre-pregnancy Maternal Weight and Gestational Weight Gain Increase the Risk for Childhood Asthma and Wheeze: An Updated Meta-Analysis

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Background: Mounting evidence suggests that childhood asthma is closely associated with maternal weight before pregnancy and gestational weight gain (GWG), yet the results are not often reproducible.

Objectives: We conducted a comprehensive meta-analysis, aiming to evaluate the association of pre-pregnancy maternal obesity or overweight and high GWG with the risk for childhood asthma and wheeze.

Methods: Literature search, quality assessment, and data extraction were completed independently and in duplicate. Effect-size estimates are expressed as odds ratio (OR) with 95% confidence interval (CI).

Results: Twenty-two observational studies involving 145,574 mother-child pairs were meta-analyzed. In overall analyses, maternal obesity or overweight in pre-pregnancy significantly increased the risk of both childhood asthma and wheeze (adjusted OR: 1.41 and 1.13, 95% CI: 1.26–1.59 and 1.07–1.20, both $p < 0.001$). Per 1 kg/m^2 increment in maternal body mass index was associated with a significantly increased risk of childhood asthma and wheeze (adjusted OR: 1.03, 95% CI: 1.02-1.03, $p < 0.001$). Compared with normal GWG, very high GWG (adjusted OR: 1.24, 95% CI: 1.04-1.47, p: 0.018), moderate high GWG (adjusted OR: 1.12, 95% CI: 1.04-1.21, p: 0.004), and very low GWG (adjusted OR: 1.26, 95% CI: 1.08-1.47, p: 0.004) increased the risk of childhood asthma and wheeze. There was a low probability of publication bias.

Conclusions: Our findings indicate that both pre-pregnancy maternal obesity or overweight and very to moderate high or low GWG render their offspring susceptible to a significantly increased risk of having childhood asthma and wheeze.

Keywords: asthma, gestational weight gain, meta-analysis, maternal obesity, maternal overweight

INTRODUCTION

Childhood asthma is the most common chronic respiratory disease, and it has reached epidemic proportions worldwide (1). Global statistics show that death rates from asthma in children range from 0 to 0.7 per 100,000 people (1). As reported by Loftus and Wise, 8.4% of persons in the United States have asthma as compared with 4.3% of the population worldwide, and both numbers are on the rise (2). It is worth noting that the average annual asthma prevalence is higher in children (9.5%) than adults (7.7%) (2). Thus, new tools to early identify children who are at risk for asthma development and could be targeted for preventive strategies are imperative to improve global health.

Evidence is mounting suggesting that childhood asthma is closely associated with maternal weight before pregnancy and gestational weight gain (GWG) (3, 4). Pre-pregnancy maternal obesity is increasingly recognized as a major public health issue worldwide. For instance, epidemiological data from the United States recorded that the prevalence of pre-pregnancy maternal obesity was 13% in 1993 and 22% in 2003, which reflects a roughly 69% increase (5). Numerous studies have examined the association between intrauterine exposure to maternal obesity or gestational weight gain and the risk of childhood asthma, with inconsistent and inconclusive findings (6–8). Forno and colleagues in 2014 interrogated summary data from 14 studies, and found that pre-pregnancy maternal obesity was associated with the significant risk of both ever and current asthma or wheeze in children, and significance was only noticed for the association between high GWG and ever asthma or wheeze (9). Given the accumulating data afterwards (4, 7, 10–15), there is a need to reevaluate this association in a more comprehensive manner.

To yield more information, we conducted an updated meta-analysis to test the hypothesis that pre-pregnancy maternal obesity or overweight and high GWG are associated with an increased risk of asthma and wheeze in children, and meanwhile we attempted to explore the possible causes of between-study heterogeneity via subgroup and meta-regression analyses.

METHODS

This meta-analysis of the available literature was conducted according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) statement (16). The PRISMA checklist is presented in **Supplementary Table 1**.

Search Strategy

The PubMed, Excerpt Medica Database (EMBASE), Cochrane Central Register of Controlled Trials (CENTRAL), Cochrane Database of Systematic Reviews, and Google Scholar were searched from inception to July 30, 2019 for observational studies that assessed the association between pre-pregnancy maternal obesity or overweight and GWG and the risk for asthma or wheeze in children.

The following medical subject headings (MESH) were used for literature search, and they are expressed in the Boolean form: (maternal OR mother OR parental OR pre-pregnancy OR pregnancy OR gestational) AND (obesity OR obese OR overweight OR body mass index OR BMI OR percent body fat OR PBF OR body weight OR anthropometry OR fat OR fatness OR adiposity OR weight gain OR body fat OR body fat composition) AND (child OR children OR childhood OR infant OR adolescent OR young OR youth OR teenage) AND (asthma OR wheeze OR asthma like symptoms OR atopic disease OR atopy OR airway hyperresponsiveness OR respiratory symptom). The bibliographies of identified articles were also searched for additional references.

Eligible Criteria

Studies were included if they fulfilled the following criteria: (i) study design: observational studies of either nested or cross-sectional case-control design; (ii) study participants: children with outcomes measured from birth to under 18 years of age, and mothers with body mass index (BMI) reported at the beginning of pregnancy or at certain point during pregnancy or with GWG reported at the end of pregnancy; (iii) study outcomes: asthma and/or wheeze; (iv) diagnoses: parental report or doctor diagnosis or medical records; (v) language: articles published in the English language. In case of study outcomes recorded at multiple time points, only outcome at the latest time point was abstracted. In a nested case-control study, cases of a disease that occur in a defined cohort are identified as the "case" group, and, for each case, a specified number of matched controls are selected from among those in the cohort who have not developed the disease by the time of disease occurrence in the case as the "control" group (17).

Articles were excluded if they were published in form of conference abstract, case report, case series, letter to the editor or correspondence, editorial, or review.

Data Extraction

The eligibility of each retrieved article was independently evaluated by two authors (S.L. and B.Z.) according to the afore mentioned inclusion and exclusion criteria, and disagreement was adjudicated by a third author (Z.Z.). The same two authors extracted data from qualified articles independently, and typed them into separate databases, including surname of the first author, year of publication, country where the study was conducted, study type, sample size, study outcomes, diagnoses of asthma and/or wheeze, effect size estimates (both adjusted and unadjusted), and baseline characteristics of study children if available, with any discrepancies resolved through discussion. The inter-rater agreement was high as revealed by the kappa statistic, which equaled to 0.99.

QUALITY ASSESSMENT

The Newcastle-Ottawa Scale (NOS) (18) was adopted for quality assessment of each qualified study. In brief, the NOS contains eight items, which are categorized into three dimensions, including selection, comparability, and outcome or exposure. The NOS ranges from zero to nine stars, with more stars indicating higher quality. Study quality was independently

assessed by two authors (S.L. and B.Z.), and areas of disagreement or uncertainty were resolved by consensus.

STATISTICAL ANALYSES

Only outcomes of interest provided by two or more studies are pooled and presented. Categorical BMI included obesity (BMI >30 kg/m^2), overweight (BMI: 25-30 kg/m^2), and underweight (BMI <18.5 kg/m^2). Categorical GWG included very low GWG (GWG <5 kg), low GWG (GWG: 5-9 kg), high GWG (GWG: 15-20 kg); moderate high GWG (GWG: 20-25 kg), and very high GWG (GWG >25 kg). Effect size estimates are presented as odds ratio (OR) with 95% confidence interval (95% CI).

Statistical heterogeneity was judged using the inconsistency index (I^2), and significant heterogeneity was reported if the I^2 is over 50% (19). In the absence of heterogeneity, fixed-effects model is adopted, and in the presence of heterogeneity, random-effects model is adopted. In case of significant heterogeneity, both fixed-effects and random-effects models yield similar results, and so random-effects model is employed irrespective of the magnitude of heterogeneity. In addition, random-effects model was used owing to assumption of clinical and methodological diversity among the studies, which often leads to statistical heterogeneity. Possible causes of between-study heterogeneity were explored by means of subgroup and meta-regression analyses.

Publication bias was visually judged by both Begg's and filled funnel plots (20). The symmetry of funnel plots was appraised by the Egger's test. The trim-and-fill method was used to estimate the number of potentially missing studies stemming from publication bias (21). Significant publication bias was recorded if the probability of the Egger's test is $<10\%$.

Statistical analyses were completed using the STATA software Release 14.1 for Windows (Stata Corp, College Station, TX).

RESULTS

Qualified Studies

Using predefined MESH terms, a total of 284 articles were identified and evaluated for eligibility. Based on the titles or abstracts, 221 articles were excluded due to obvious reasons. After reviewing the full texts of remaining 63 articles, 41 articles were further excluded, leaving 22 articles that fulfilled predetermined eligibility criteria in this meta-analysis (4, 7, 8, 10–15, 22–34). A flow diagram illustrating the exclusion of articles with specific reasons is shown in **Figure 1**.

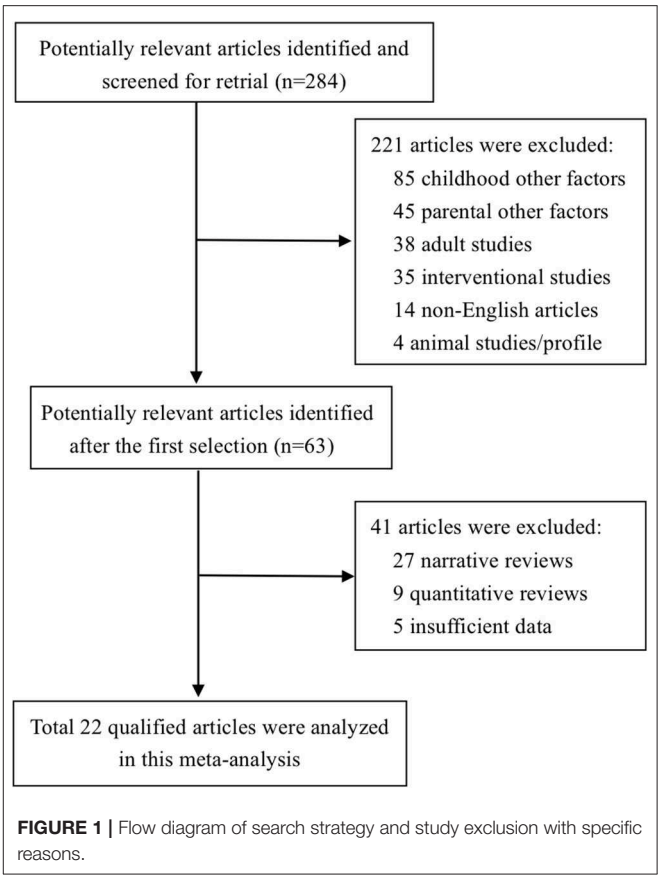

FIGURE 1 | Flow diagram of search strategy and study exclusion with specific reasons.

Baseline Characteristics of Qualified Studies

The baseline characteristics of 22 observational studies (2 cross-sectional and 20 nested case-control studies) involving 145,574 mother-child pairs were showed in **Table 1**. The average age of children ranged from 4 months to 16 years. Sixteen studies reported maternal categorical BMI before or at the beginning of pregnancy (4, 7, 8, 11–15, 24–28, 30, 32, 34), and thereof ten studies additionally reported maternal continuous BMI in pre-pregnancy (7, 10, 11, 14, 15, 27–29, 32, 33). Seven studies reported GWG, with one study reporting continuous GWG (32), and six reporting categorical GWG (4, 7, 8, 22, 23, 31). Three studies reported categorical GWG according to Institute of Medicine criteria (7, 14, 33).

Quality Assessment

Using the NOS system, the total stars of 2 cross-sectional case-control studies were 6 and 4, respectively (**Supplementary Table 2**). For nested case-control studies, the total stars ranged from 5 to 8 (mean: 6.45, standard deviation: 0.83) (**Supplementary Table 3**).

Overall Analyses

For maternal categorical BMI in pre-pregnancy, the forest plots of study outcomes before and after adjustment are shown in **Supplementary Figure 1** and **Figure 2**, respectively. Before adjustment, maternal obesity and overweight in pre-pregnancy significantly increased the risk of childhood asthma and wheeze (OR: 1.59 and 1.18, 95% CI: 1.38-1.83 and 1.06-1.3, both $p < 0.001$), with significant heterogeneity (I^2: 79.5% and 78%; $p < 0.001$ and p: 0.002). The association between pre-pregnancy maternal underweight and the risk of childhood asthma and wheeze was statistically significant (OR: 1.09, 95% CI: 1.01-1.08, $p < 0.001$), without evidence of heterogeneity (I^2: 0.0%; p: 0.486).

After adjustment, maternal obesity and overweight in pre-pregnancy significantly increased the risk of both childhood

TABLE 1 | The baseline characteristics of individual studies involved in this meta-analysis.

References	Maternal weight	Continent	Study type	Sample size	Male	Age	Weight modality	Diagnosis of asthma	Clinical endpoint	Comparison	ES	cES	aES
Oliveti et al. (26)	GWG <9 kg	America	Cross-sectional	262	0.634	6.7	Self-report	Doctor-diagnosis	Asthma	Low GWG vs. normal GWG	OR	4.4	3.42
Rusconi et al. (23)	GWG <9 kg	Europe	Cross-sectional	15,609	0.512	6.7	Self-report	Self-report	Persistent wheeze	Low GWG vs. normal GWG	OR	1.09	1.08
Rusconi et al. (23)	GWG >15 kg	Europe	Cross-sectional	15,609	0.512	6.7	Self-report	Self-report	Persistent wheeze	High GWG vs. normal GWG	OR	1.23	1.2
Reichman et al. (24)	BMI ≥30 kg/m2	America	Longitudinal	1,971	0.524	3	Medical records	Doctor-diagnosis	Asthma	High BMI vs. normal BMI	OR	1.51	1.34
Haberg et al. (25)	BMI: 25–30 kg/m2	Europe	Longitudinal	33,192	NA	1.5	Self-report	Self-report	Wheeze	High BMI vs. normal BMI	OR	0.96	1.02
Haberg et al. (25)	BMI ≥30 kg/m2	Europe	Longitudinal	33,192	NA	1.5	Self-report	Self-report	Wheeze	High BMI vs. normal BMI	OR	1.21	1.15
Kumar et al. (26)	BMI: 25–30 kg/m2	America	Longitudinal	1,191	0.483	3	Self-report	Self-report	Recurrent wheeze	High BMI vs. normal BMI	OR	1.48	1.58
Kumar et al. (26)	BMI ≥30 kg/m2	America	Longitudinal	1,191	0.483	3	Self-report	Self-report	Recurrent wheeze	High BMI vs. normal BMI	OR	3.24	3.51
Scholtens et al. (27)	Continuous BMI	Europe	Longitudinal	3,963	NA	8	Self-report	Self-report	Asthma	Per BMI increment	OR	NA	0.98
Scholtens et al. (27)	Continuous BMI	Europe	Longitudinal	3,963	NA	8	Self-report	Self-report	Wheeze	Per BMI increment	OR	NA	1.01
Scholtens et al. (27)	Continuous BMI	Europe	Longitudinal	3,963	NA	8	Self-report	Self-report	Asthma	Per BMI increment	OR	NA	1.05
Scholtens et al. (27)	Continuous BMI	Europe	Longitudinal	3,963	NA	8	Self-report	Self-report	Wheeze	Per BMI increment	OR	NA	1.06
Scholtens et al. (27)	BMI >25 kg/m2	Europe	Longitudinal	3,963	NA	8	Self-report	Self-report	Asthma	High BMI vs. normal BMI	OR	1	0.86
Scholtens et al. (27)	BMI >25 kg/m2	Europe	Longitudinal	3,963	NA	8	Self-report	Self-report	Wheeze	High BMI vs. normal BMI	OR	1.1	1.06
Scholtens et al. (27)	BMI >25 kg/m2	Europe	Longitudinal	3,963	NA	8	Self-report	Self-report	Asthma	High BMI vs. normal BMI	OR	1.7	1.52
Scholtens et al. (27)	BMI >25 kg/m2	Europe	Longitudinal	3,963	NA	8	Self-report	Self-report	Wheeze	High BMI vs. normal BMI	OR	1.58	1.44
Patel et al. (28)	BMI: 25–30 kg/m2	Europe	Longitudinal	6,945	0.515	15.5	Medical records	Doctor-diagnosis	Ever wheeze	High BMI vs. normal BMI	OR	1.31	1.18
Patel et al. (28)	BMI: 25–30 kg/m2	Europe	Longitudinal	6,945	0.515	15.5	Medical records	Doctor-diagnosis	Current wheeze	High BMI vs. normal BMI	OR	1.22	1.13
Patel et al. (28)	BMI <19 kg/m2	Europe	Longitudinal	6,945	0.515	15.5	Medical records	Doctor-diagnosis	Ever wheeze	Low BMI vs. normal BMI	OR	0.91	0.8
Patel et al. (28)	BMI <19 kg/m2	Europe	Longitudinal	6,945	0.515	15.5	Medical records	Doctor-diagnosis	Current wheeze	Low BMI vs. normal BMI	OR	1.07	0.87
Patel et al. (28)	BMI ≥30 kg/m2	Europe	Longitudinal	6,945	0.515	15.5	Medical records	Doctor-diagnosis	Ever wheeze	High BMI vs. normal BMI	OR	1.07	0.99
Patel et al. (28)	BMI ≥30 kg/m2	Europe	Longitudinal	6,945	0.515	15.5	Medical records	Doctor-diagnosis	Current wheeze	High BMI vs. normal BMI	OR	1.52	1.54
Patel et al. (28)	Continuous BMI	Europe	Longitudinal	6,945	0.515	15.5	Medical records	Doctor-diagnosis	Ever wheeze	Per BMI increment	OR	1.03	1.03
Patel et al. (28)	Continuous BMI	Europe	Longitudinal	6,945	0.515	15.5	Medical records	Doctor-diagnosis	Current wheeze	Per BMI increment	OR	1.04	1.05
Caudri et al. (29)	Continuous BMI	Europe	Longitudinal	3,963	0.516	8	Self-report	Self-report	Persistent wheeze	Per BMI increment	OR	NA	1.06
Guerra et al. (30)	BMI: 25–30 kg/m2	Europe	Longitudinal	1,107	0.511	1.17	Self-report	Self-report	Persistent wheeze	High BMI vs. normal BMI	RR	1.3	1
Guerra et al. (30)	BMI <19 kg/m2	Europe	Longitudinal	1,107	0.511	1.17	Self-report	Self-report	Persistent wheeze	Low BMI vs. normal BMI	RR	1.5	1.7
Guerra et al. (30)	BMI ≥30 kg/m2	Europe	Longitudinal	1,107	0.511	1.17	Self-report	Self-report	Persistent wheeze	High BMI vs. normal BMI	RR	4.1	4.2
Halonen et al. (31)	GWG >18.6 kg	America	Longitudinal	261	NA	9	Medical records	Doctor-diagnosis	Asthma	High GWG vs. normal GWG	OR	3.4	3.4
Halonen et al. (31)	BMI <18.5 kg/m2	Europe	Longitudinal	38,874	0.51	7	Self-report	Doctor-diagnosis	Asthma	Low BMI vs. normal BMI	OR	1.11	1.03
Halonen et al. (31)	BMI <18.5 kg/m2	Europe	Longitudinal	38,874	0.51	7	Self-report	Doctor-diagnosis	Ever asthma	Low BMI vs. normal BMI	OR	NA	1.02
Halonen et al. (31)	BMI <18.5 kg/m2	Europe	Longitudinal	38,874	0.51	7	Self-report	Doctor-diagnosis	Current asthma	Low BMI vs. normal BMI	OR	NA	1.07
Halonen et al. (31)	BMI <18.5 kg/m2	Europe	Longitudinal	38,874	0.51	7	Self-report	Doctor-diagnosis	Persistent wheeze	Low BMI vs. normal BMI	OR	0.91	0.81
Halonen et al. (31)	BMI: 25–30 kg/m2	Europe	Longitudinal	38,874	0.51	7	Self-report	Doctor-diagnosis	Ever asthma	High BMI vs. normal BMI	OR	NA	1.19
Halonen et al. (31)	BMI: 25–30 kg/m2	Europe	Longitudinal	38,874	0.51	7	Self-report	Doctor-diagnosis	Current asthma	High BMI vs. normal BMI	OR	NA	1.24
Halonen et al. (31)	BMI: 30–35 kg/m2	Europe	Longitudinal	38,874	0.51	7	Self-report	Doctor-diagnosis	Persistent wheeze	High BMI vs. normal BMI	OR	1.78	1.62
Halonen et al. (31)	BMI: 25–30 kg/m2	Europe	Longitudinal	38,874	0.51	7	Self-report	Doctor-diagnosis	Persistent wheeze	High BMI vs. normal BMI	OR	1.28	1.19

(Continued)

TABLE 1 | Continued

References	Maternal weight	Continent	Study type	Sample size	Male	Age	Weight modality	Diagnosis of asthma	Clinical endpoint	Comparison	ES	cES	aES
Halonen et al. (31)	BMI: 25–30 kg/m2	Europe	Longitudinal	38,874	0.51	7	Self-report	Doctor-diagnosis	Asthma	High BMI vs. normal BMI	OR	1.24	1.22
Halonen et al. (31)	BMI: 30–35 kg/m2	Europe	Longitudinal	38,874	0.51	7	Self-report	Doctor-diagnosis	Asthma	High BMI vs. normal BMI	OR	1.6	1.56
Halonen et al. (31)	BMI: 30–35 kg/m2	Europe	Longitudinal	38,874	0.51	7	Self-report	Doctor-diagnosis	Ever asthma	High BMI vs. normal BMI	OR	NA	1.5
Halonen et al. (31)	BMI: 30–35 kg/m2	Europe	Longitudinal	38,874	0.51	7	Self-report	Doctor-diagnosis	Current asthma	High BMI vs. normal BMI	OR	NA	1.58
Halonen et al. (31)	BMI ≥35 kg/m2	Europe	Longitudinal	38,874	0.51	7	Self-report	Doctor-diagnosis	Asthma	High BMI vs. normal BMI	OR	1.64	1.55
Halonen et al. (31)	BMI ≥35 kg/m2	Europe	Longitudinal	38,874	0.51	7	Self-report	Doctor-diagnosis	Ever asthma	High BMI vs. normal BMI	OR	NA	1.55
Halonen et al. (31)	BMI ≥35 kg/m2	Europe	Longitudinal	38,874	0.51	7	Self-report	Doctor-diagnosis	Current asthma	High BMI vs. normal BMI	OR	NA	1.48
Halonen et al. (31)	BMI ≥35 kg/m2	Europe	Longitudinal	38,874	0.51	7	Self-report	Doctor-diagnosis	Persistent wheeze	High BMI vs. normal BMI	OR	1.6	1.44
Halonen et al. (31)	GWG: 16–19 kg	Europe	Longitudinal	38,874	0.51	7	Self-report	Doctor-diagnosis	Asthma	High GWG vs. normal GWG	OR	0.96	0.97
Halonen et al. (31)	GWG: 16–19 kg	Europe	Longitudinal	38,874	0.51	7	Self-report	Doctor-diagnosis	Ever asthma	High GWG vs. normal GWG	OR	NA	1.03
Halonen et al. (31)	GWG: 16–19 kg	Europe	Longitudinal	38,874	0.51	7	Self-report	Doctor-diagnosis	Current asthma	High GWG vs. normal GWG	OR	NA	0.9
Halonen et al. (31)	GWG: 16–19 kg	Europe	Longitudinal	38,874	0.51	7	Self-report	Doctor-diagnosis	Persistent wheeze	High GWG vs. normal GWG	OR	0.96	0.99
Halonen et al. (31)	GWG: 20–24 kg	Europe	Longitudinal	38,874	0.51	7	Self-report	Doctor-diagnosis	Asthma	Moderate high GWG vs. normal GWG	OR	1.18	1.15
Halonen et al. (31)	GWG: 20–24 kg	Europe	Longitudinal	38,874	0.51	7	Self-report	Doctor-diagnosis	Ever asthma	Moderate high GWG vs. normal GWG	OR	NA	1.13
Halonen et al. (31)	GWG: 20–24 kg	Europe	Longitudinal	38,874	0.51	7	Self-report	Doctor-diagnosis	Current asthma	Moderate high GWG vs. normal GWG	OR	NA	1.15
Halonen et al. (31)	GWG: 20–24 kg	Europe	Longitudinal	38,874	0.51	7	Self-report	Doctor-diagnosis	Persistent wheeze	Moderate high GWG vs. normal GWG	OR	1	0.96
Halonen et al. (31)	GWG: 5–9 kg	Europe	Longitudinal	38,874	0.51	7	Self-report	Doctor-diagnosis	Asthma	Low GWG vs. normal GWG	OR	1.13	1.02
Halonen et al. (31)	GWG: 5–9 kg	Europe	Longitudinal	38,874	0.51	7	Self-report	Doctor-diagnosis	Ever asthma	Low GWG vs. normal GWG	OR	NA	0.94
Halonen et al. (31)	GWG: 5–9 kg	Europe	Longitudinal	38,874	0.51	7	Self-report	Doctor-diagnosis	Current asthma	Low GWG vs. normal GWG	OR	NA	1.11
Halonen et al. (31)	GWG: 5–9 kg	Europe	Longitudinal	38,874	0.51	7	Self-report	Doctor-diagnosis	Persistent wheeze	Low GWG vs. normal GWG	OR	1.41	1.24
Halonen et al. (31)	GWG <5 kg	Europe	Longitudinal	38,874	0.51	7	Self-report	Doctor-diagnosis	Asthma	Very low GWG vs. normal GWG	OR	1.62	1.17
Halonen et al. (31)	GWG <5 kg	Europe	Longitudinal	38,874	0.51	7	Self-report	Doctor-diagnosis	Ever asthma	Very low GWG vs. normal GWG	OR	NA	1.54
Halonen et al. (31)	GWG <5 kg	Europe	Longitudinal	38,874	0.51	7	Self-report	Doctor-diagnosis	Current asthma	Very low GWG vs. normal GWG	OR	NA	0.84
Halonen et al. (31)	GWG <5 kg	Europe	Longitudinal	38,874	0.51	7	Self-report	Doctor-diagnosis	Persistent wheeze	Very low GWG vs. normal GWG	OR	1.2	0.82
Halonen et al. (31)	GWG ≥25 kg	Europe	Longitudinal	38,874	0.51	7	Self-report	Doctor-diagnosis	Asthma	Very high GWG vs. normal GWG	OR	1.27	1.19
Halonen et al. (31)	GWG ≥25 kg	Europe	Longitudinal	38,874	0.51	7	Self-report	Doctor-diagnosis	Ever asthma	Very high GWG vs. normal GWG	OR	NA	1.11
Halonen et al. (31)	GWG ≥25 kg	Europe	Longitudinal	38,874	0.51	7	Self-report	Doctor-diagnosis	Current asthma	Very high GWG vs. normal GWG	OR	NA	1.23
Halonen et al. (31)	GWG ≥25 kg	Europe	Longitudinal	38,874	0.51	7	Self-report	Doctor-diagnosis	Persistent wheeze	Very high GWG vs. normal GWG	OR	1.19	1.12

(Continued)

TABLE 1 | Continued

References	Maternal weight	Continent	Study type	Sample size	Male	Age	Weight modality	Diagnosis of asthma	Clinical endpoint	Comparison	ES	cES	aES
Leermakers et al. (32)	BMI: 25–30 kg/m2	Europe	Longitudinal	4,656	0.5	4	Self-report	Self-report	Wheeze	High BMI vs. normal BMI	OR	NA	1.04
Leermakers et al. (32)	BMI: 25–30 kg/m2	Europe	Longitudinal	4,656	0.5	4	Self-report	Self-report	Wheeze	High BMI vs. normal BMI	OR	NA	1.09
Leermakers et al. (32)	BMI: 25–30 kg/m2	Europe	Longitudinal	4,656	0.5	4	Self-report	Self-report	Wheeze	High BMI vs. normal BMI	OR	NA	0.95
Leermakers et al. (32)	BMI <20 kg/m2	Europe	Longitudinal	4,656	0.5	4	Self-report	Self-report	Wheeze	Low BMI vs. normal BMI	OR	NA	1.02
Leermakers et al. (32)	BMI <20 kg/m2	Europe	Longitudinal	4,656	0.5	4	Self-report	Self-report	Wheeze	Low BMI vs. normal BMI	OR	NA	0.93
Leermakers et al. (32)	BMI <20 kg/m2	Europe	Longitudinal	4,656	0.5	4	Self-report	Self-report	Wheeze	Low BMI vs. normal BMI	OR	NA	1.19
Leermakers et al. (32)	BMI ≥30 kg/m2	Europe	Longitudinal	4,656	0.5	4	Self-report	Self-report	Wheeze	High BMI vs. normal BMI	OR	NA	1.1
Leermakers et al. (32)	BMI ≥30 kg/m2	Europe	Longitudinal	4,656	0.5	4	Self-report	Self-report	Wheeze	High BMI vs. normal BMI	OR	NA	0.93
Leermakers et al. (32)	BMI ≥30 kg/m2	Europe	Longitudinal	4,656	0.5	4	Self-report	Self-report	Wheeze	High BMI vs. normal BMI	OR	NA	1.41
Leermakers et al. (32)	Continuous BMI	Europe	Longitudinal	4,656	0.5	4	Self-report	Self-report	Wheeze	Per BMI increment	OR	NA	1.01
Leermakers et al. (32)	Continuous BMI	Europe	Longitudinal	4,656	0.5	4	Self-report	Self-report	Wheeze	Per BMI increment	OR	NA	1.01
Pike et al. (33)	Continuous BMI	Europe	Longitudinal	940	0.516	6	Medical records	Both	Ever wheeze	Per BMI increment	RR	1.02	1.01
Pike et al. (33)	Continuous BMI	Europe	Longitudinal	940	0.516	6	Medical records	Both	Ever asthma	Per BMI increment	RR	1.01	1
Pike et al. (33)	Continuous BMI	Europe	Longitudinal	940	0.516	6	Medical records	Both	Current wheeze	Per BMI increment	RR	1	1
Pike et al. (33)	Continuous BMI	Europe	Longitudinal	940	0.516	6	Medical records	Both	Current asthma	Per BMI increment	RR	1.02	1.02
Pike et al. (33)	Continuous BMI	Europe	Longitudinal	940	0.516	6	Medical records	Both	Persistent wheeze	Per BMI increment	RR	1.02	1.02
Pike et al. (33)	Excessive	Europe	Longitudinal	940	0.516	6	Medical records or Measure	Both	Ever wheeze	High GWG vs. normal GWG	RR	NA	0.96
Pike et al. (33)	Excessive	Europe	Longitudinal	940	0.516	6	Medical records or Measure	Both	Ever asthma	High GWG vs. normal GWG	RR	NA	0.99
Pike et al. (33)	Excessive	Europe	Longitudinal	940	0.516	6	Medical records or Measure	Both	Current wheeze	High GWG vs. normal GWG	RR	NA	0.83
Pike et al. (33)	Excessive	Europe	Longitudinal	940	0.516	6	Medical records or Measure	Both	Current asthma	High GWG vs. normal GWG	RR	NA	1.05
Pike et al. (33)	Excessive	Europe	Longitudinal	940	0.516	6	Medical records or Measure	Both	Persistent wheeze	High GWG vs. normal GWG	RR	NA	0.94
Pike et al. (33)	Inadequate	Europe	Longitudinal	940	0.516	6	Medical records or Measure	Both	Ever wheeze	Low GWG vs. normal GWG	RR	NA	0.98
Pike et al. (33)	Inadequate	Europe	Longitudinal	940	0.516	6	Medical records or Measure	Both	Ever asthma	Low GWG vs. normal GWG	RR	NA	1.15
Pike et al. (33)	Inadequate	Europe	Longitudinal	940	0.516	6	Medical records or Measure	Both	Current wheeze	Low GWG vs. normal GWG	RR	NA	0.80
Pike et al. (33)	Inadequate	Europe	Longitudinal	940	0.516	6	Medical records or Measure	Both	Current asthma	Low GWG vs. normal GWG	RR	NA	1.24
Pike et al. (33)	Inadequate	Europe	Longitudinal	940	0.516	6	Medical records or Measure	Both	Persistent wheeze	Low GWG vs. normal GWG	RR	NA	0.91
Wright et al. (33)	BMI ≥30 kg/m2	America	Longitudinal	261	0.49	2	Medical records	Self-report	Recurrent wheeze	High BMI vs. normal BMI	OR	2.61	2.56
de Vries et al. (10)	Continuous BMI	Europe	Longitudinal	4,860	0.499	0.28	Self-report	Self-report	Wheeze	Per BMI increment	OR	1.03	1.03

(Continued)

TABLE 1 | Continued

References	Maternal weight	Continent	Study type	Sample size	Male	Age	Weight modality	Diagnosis of asthma	Clinical endpoint	Comparison	ES	cES	aES
Ekstrom et al. (11)	BMI: 25–29.9 kg/m2	Europe	Longitudinal	3,294	0.505	16	Medical records	Self-report	Asthma	High BMI vs. normal BMI	OR	NA	1.14
Ekstrom et al. (11)	BMI <18.5 kg/m2	Europe	Longitudinal	3,294	0.505	16	Medical records	Self-report	Asthma	Low BMI vs. normal BMI	OR	NA	1.29
Ekstrom et al. (11)	BMI ≥30 kg/m2	Europe	Longitudinal	3,294	0.505	16	Medical records	Self-report	Asthma	High BMI vs. normal BMI	OR	NA	1.53
Ekstrom et al. (11)	Continuous BMI	Europe	Longitudinal	3,294	0.505	16	Medical records	Self-report	Asthma	Per BMI increment	OR	NA	1.06
Harskamp-van et al. (15)	BMI: 25–29.9 kg/m2	Europe	Longitudinal	3,185	NA	7.5	Self-report	Doctor-diagnosis	Current wheeze	High BMI vs. normal BMI	RR	0.93	0.9
Harskamp-van et al. (15)	BMI: 25–29.9 kg/m2	Europe	Longitudinal	3,185	NA	7.5	Self-report	Doctor-diagnosis	Ever asthma	High BMI vs. normal BMI	RR	1.33	1.38
Harskamp-van et al. (15)	BMI <18.5 kg/m2	Europe	Longitudinal	3,185	NA	7.5	Self-report	Doctor-diagnosis	Current wheeze	Low BMI vs. normal BMI	RR	1.15	1.32
Harskamp-van et al. (15)	BMI <18.5 kg/m2	Europe	Longitudinal	3,185	NA	7.5	Self-report	Doctor-diagnosis	Ever asthma	Low BMI vs. normal BMI	RR	1.64	1.42
Harskamp-van et al. (15)	BMI ≥30 kg/m2	Europe	Longitudinal	3,185	NA	7.5	Self-report	Doctor-diagnosis	Current wheeze	High BMI vs. normal BMI	RR	2.09	2.15
Harskamp-van et al. (15)	BMI ≥30 kg/m2	Europe	Longitudinal	3,185	NA	7.5	Self-report	Doctor-diagnosis	Ever asthma	High BMI vs. normal BMI	RR	2.72	2.24
Dumas et al. (4)	BMI: 25–29.9 kg/m2	America	Longitudinal	12,963	0.462	11.5	Self-report	Doctor-diagnosis	Asthma	High BMI vs. normal BMI	OR	1.27	1.19
Dumas et al. (4)	BMI <20.0 kg/m2	America	Longitudinal	12,963	0.462	11.5	Self-report	Doctor-diagnosis	Asthma	Low BMI vs. normal BMI	OR	1.08	1.05
Dumas et al. (4)	BMI ≥30 kg/m2	America	Longitudinal	12,963	0.462	11.5	Self-report	Doctor-diagnosis	Asthma	High BMI vs. normal BMI	OR	1.48	1.34
Dumas et al. (4)	GWG: 15.9–20 kg	America	Longitudinal	12,963	0.462	11.5	Self-report	Doctor-diagnosis	Asthma	High GWG vs. normal GWG	OR	1.09	1.04
Dumas et al. (4)	GWG: 6.8–10.9 kg	America	Longitudinal	12,963	0.462	11.5	Self-report	Doctor-diagnosis	Asthma	Low GWG vs. normal GWG	OR	1.08	1.07
Dumas et al. (4)	GWG <6.8 kg	America	Longitudinal	12,963	0.462	11.5	Self-report	Doctor-diagnosis	Asthma	Very low GWG vs. normal GWG	OR	1.51	1.28
Dumas et al. (4)	GWG ≥20.4 kg	America	Longitudinal	12,963	0.462	11.5	Self-report	Doctor-diagnosis	Asthma	Moderate high GWG vs. normal GWG	OR	1.16	1.05
Taylor-Robinson et al. (13)	Obesity	Europe	Longitudinal	11,141	0.509	7	Self-report	Self-report	Persistent wheeze	High BMI vs. normal BMI	RRR	1.38	1.27
Taylor-Robinson et al. (13)	Overweight	Europe	Longitudinal	11,141	0.509	7	Self-report	Self-report	Persistent wheeze	High BMI vs. normal BMI	RRR	1.25	1.22
Taylor-Robinson et al. (13)	Underweight	Europe	Longitudinal	11,141	0.509	7	Self-report	Self-report	Persistent wheeze	Low BMI vs. normal BMI	RRR	1.1	1.02
Polinski et al. (7)	BMI: 25–29.9 kg/m2	America	Longitudinal	6,450	0.489	4	Self-report	Doctor-diagnosis	Asthma	High BMI vs. normal BMI	OR	1.26	1.22
Polinski et al. (7)	BMI <18.5 kg/m2	America	Longitudinal	6,450	0.489	4	Self-report	Doctor-diagnosis	Asthma	Low BMI vs. normal BMI	OR	1.18	1.07
Polinski et al. (7)	BMI ≥30 kg/m2	America	Longitudinal	6,450	0.489	4	Self-report	Doctor-diagnosis	Asthma	High BMI vs. normal BMI	OR	1.8	1.5
Polinski et al. (7)	Continuous BMI	America	Longitudinal	6,450	0.489	4	Self-report	Doctor-diagnosis	Asthma	Per BMI increment	OR	1.03	1.03
Polinski et al. (7)	GWG: 16–19 kg	America	Longitudinal	6,450	0.489	4	Self-report	Doctor-diagnosis	Asthma	High GWG vs. normal GWG	OR	0.79	0.84
Polinski et al. (7)	GWG: 20–24 kg	America	Longitudinal	6,450	0.489	4	Self-report	Doctor-diagnosis	Asthma	Moderate high GWG vs. normal GWG	OR	1.14	1.16
Polinski et al. (7)	GWG: 5–9 kg	America	Longitudinal	6,450	0.489	4	Self-report	Doctor-diagnosis	Asthma	Low GWG vs. normal GWG	OR	1.25	1.06

(Continued)

TABLE 1 | Continued

References	Maternal weight	Continent	Study type	Sample size	Male	Age	Weight modality	Diagnosis of asthma	Clinical endpoint	Comparison	ES	cES	aES
Polinski et al. (7)	GWG <5 kg	America	Longitudinal	6,450	0.489	4	Self-report	Doctor-diagnosis	Asthma	Very low GWG vs. normal GWG	OR	2.2	1.56
Polinski et al. (7)	GWG ≥25 kg	America	Longitudinal	6,450	0.489	4	Self-report	Doctor-diagnosis	Asthma	Very high GWG vs. normal GWG	OR	1.71	1.53
Polinski et al. (7)	Excessive	America	Longitudinal	6,450	0.489	4	Self-report	Doctor-diagnosis	Asthma	High GWG vs. normal GWG	OR	0.95	0.85
Polinski et al. (7)	Inadequate	America	Longitudinal	6,450	0.489	4	Self-report	Doctor-diagnosis	Asthma	Low GWG vs. normal GWG	OR	1.13	0.99
Rajappan et al. (14)	BMI <18.5 kg/m2	Europe	Longitudinal	2,799	0.516	1	Medical records	Self-report	Wheeze	Low BMI vs. normal BMI	RR	0.7	0.71
Rajappan et al. (14)	BM ≥30 kg/m2	Europe	Longitudinal	2,799	0.516	1	Medical records	Self-report	Wheeze	High BMI vs. normal BMI	RR	1.19	1.19
Rajappan et al. (14)	BMI: 25–29.99 kg/m2	Europe	Longitudinal	2,799	0.516	1	Medical records	Self-report	Wheeze	High BMI vs. normal BMI	RR	1.08	1.11
Rajappan et al. (14)	Continuous BMI	Europe	Longitudinal	2,799	0.516	1	Medical records	Self-report	Wheeze	Per BMI increment	RR	1.02	1.02
Rajappan et al. (14)	Excessive	Europe	Longitudinal	2,799	0.516	1	Medical records or Measure	Self-report	Wheeze	High GWG vs. normal GWG	RR	NA	0.97
Rajappan et al. (14)	Inadequate	Europe	Longitudinal	2,799	0.516	1	Medical records or Measure	Self-report	Wheeze	Low GWG vs. normal GWG	RR	NA	1.05
Goudarzi et al. (15)	BMI: 25–29.9 kg/m2	Asia	Longitudinal	3,296	0.516	7	Self-report	Self-report	Wheeze	High BMI vs. normal BMI	RR	NA	1.11
Goudarzi et al. (15)	BMI <18.5 kg/m2	Asia	Longitudinal	3,296	0.516	7	Self-report	Self-report	Wheeze	Low BMI vs. normal BMI	RR	NA	0.69
Goudarzi et al. (15)	BMI ≥30 kg/m2	Asia	Longitudinal	3,296	0.516	7	Self-report	Self-report	Wheeze	High BMI vs. normal BMI	RR	NA	1.67
Goudarzi et al. (15)	Continuous BMI	Asia	Longitudinal	3,296	0.516	7	Self-report	Self-report	Wheeze	Per BMI increment	RR	1.03	1.03

ES, effect size; cES, crude effect size; aES, adjusted effect size; GWG, gestational weight gain; BMI, body mass index; NA, not available.

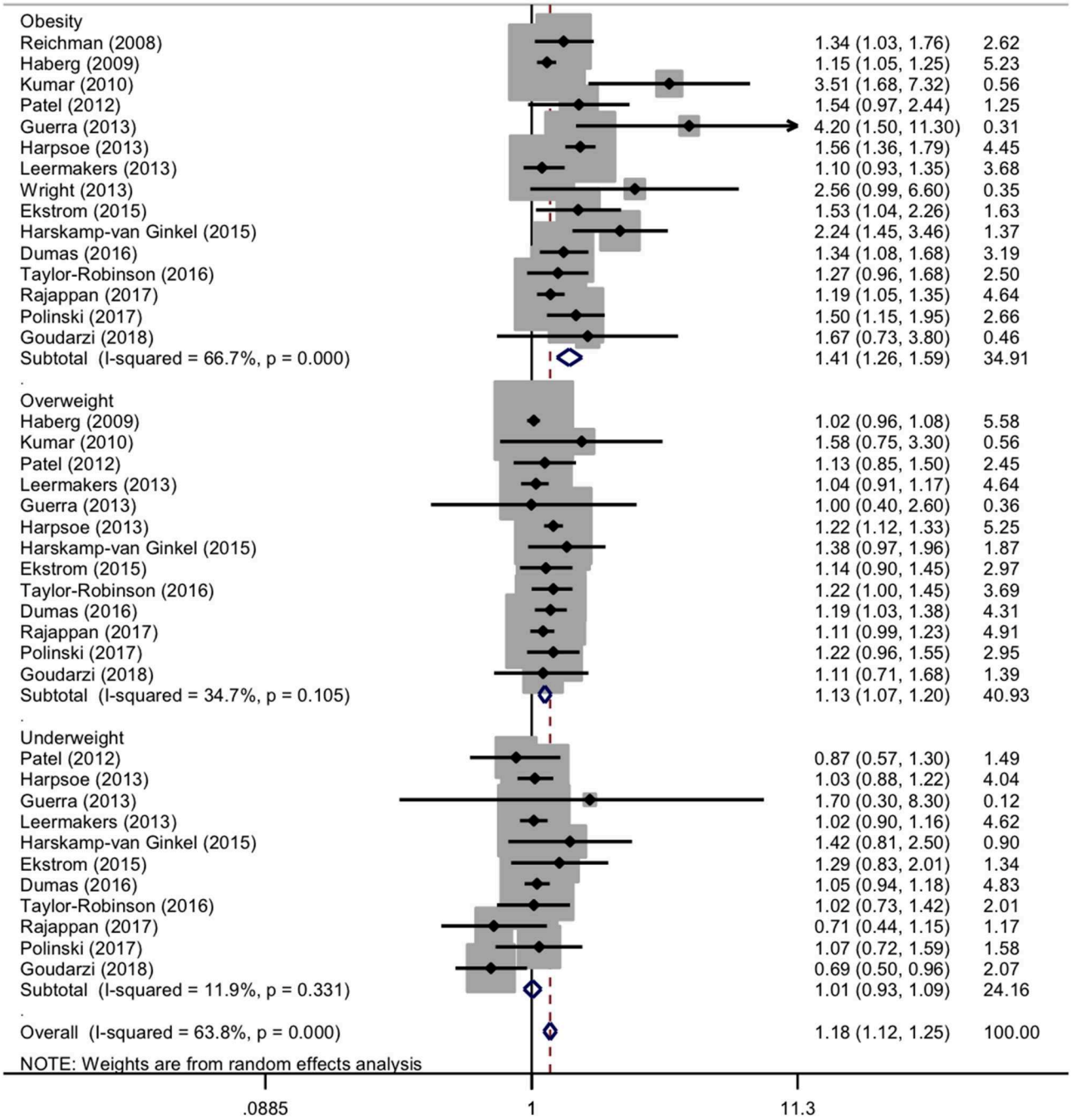

FIGURE 2 | Forest plots for categorical body mass index after adjustment. OR, odds ratio; 95% CI, 95% confidence interval. The gray shadow size represents the proportion of the weight. The black line equal to 1 perpendicular to the horizontal axis represents an invalid line, and the red dashed line parallel to the black line represents the combined effect line of all the included studies.

asthma and wheeze (OR: 1.41 and 1.13, 95% CI: 1.26-1.59 and 1.07-1.20, both $p < 0.001$), with moderate and low evidence of heterogeneity (I^2: 66.7% and 34.7%; $p < 0.001$ and p: 0.105). Contrastingly, the association between maternal underweight in pre-pregnancy and the risk of childhood asthma and wheeze was nonsignificant (OR: 1.01, 95% CI: 0.93-1.09, p: 0.799), without heterogeneity (I^2: 11.9%; p: 0.331).

For maternal continuous BMI in pre-pregnancy, the forest plots of study outcomes before and after adjustment are showed in **Supplementary Figure 2** and **Figure 3**, respectively. Per 1 kg/m^2 increment in maternal BMI was associated with a significantly increased risk of childhood asthma and wheeze before (OR: 1.03, 95% CI: 1.02-1.03, $p < 0.001$) and after (OR: 1.03, 95% CI: 1.02-1.03, $p < 0.001$) adjustment, with none and low evidence of heterogeneity (I^2: 0.0% and 31.9%; p: 0.676 and 0.153, respectively).

For categorical GWG in pre-pregnancy, the forest plots of study outcomes before and after adjustment are shown in **Supplementary Figure 3** and **Figure 4**, respectively. Before and after adjustment, the risk of childhood asthma and wheeze was significantly increased for very high GWG (OR: 1.38 and 1.24, 95% CI: 1.06-1.80 and 1.04-1.47, p: 0.016 and 0.018, I^2: 47.4 and 18%), moderate high GWG (OR: 1.17 and 1.12, 95% CI: 1.08-1.26 and 1.04-1.21, $p < 0.001$ and p: 0.004, both I^2: 0.0%), and very low GWG (OR: 1.67 and 1.26, 95% CI: 1.46-1.99 and 1.08-1.47, $p < 0.001$ and p: 0.004, I^2: 27.1% and 0.0%), as compared with normal GWG. By contrast, high GWG was not associated with a significantly increased risk of childhood asthma and wheeze before (OR: 1.09, 95% CI: 0.90-1.31, p: 0.357, I^2: 79.3%) and after (OR: 1.07, 95% CI:0.91-1.25, p: 0.493, I^2: 73.7%) adjustment. Besides, there was significant association between low GWG (OR: 1.24, 95% CI: 1.03-1.50, p: 0.027, I^2: 78.3%) and risk of asthma and wheeze in childhood before adjustment, yet no significant (OR: 1.11, 95% CI: 0.95-1.29, p: 0.182, I^2: 65.6%) after adjustment. According to the Institute of Medicine criteria for GWG, the forest plots of study outcomes after adjustment are shown in **Figure 5**. Compared with adequate GWG, the risk of childhood asthma and wheeze was not significantly associated with inadequate GWG (OR: 0.95, 95% CI: 0.86-1.05, p: 0.44, I^2: 0.0%), excessive GWG (OR: 0.99, 95% CI: 0.92-1.07, p: 0.31, I^2: 0.0%).

Subgroup Analyses

Due to significantly statistical heterogeneity encountered in overall analysis, several subgroup analyses were conducted separately according to sample size, region, weight modality, and diagnosis of asthma for maternal pre-pregnancy BMI (categorical and continuous) before (**Supplementary Tables 4**, **5**) and after adjustment (**Table 2** and **Supplementary Table 6**).

With regard to sample size, pre-pregnancy maternal obesity was associated with 50% increased risk of childhood asthma in studies with sample size ≥6000 (adjusted OR: 1.50, 95% CI: 1.34-1.66, $p < 0.001$), which was lower than studies with sample size <6000 (adjusted OR: 1.60, 95% CI: 1.20-2.13, p: 0.001) (**Table 2**). The case was similar for the risk of childhood wheezing (adjusted OR: 1.28 and 1.68, 95% CI: 1.25-2.26 and 1.10-149, both p: 0.001 for studies with sample size ≥6000 and <6000, respectively) (**Table 2**).

By diagnosis of asthma, pre-pregnancy maternal overweight was respectively associated with 22 and 14% increased risk of doctor-diagnosed asthma (adjusted OR: 1.22, 95% CI: 1.14-1.31, $p < 0.001$) and parent-reported asthma (adjusted OR: 1.14, 95% CI: 0.86-1.51, $p < 0.362$) (**Table 2**). By weight modality, the more significantly increased risk of asthma in childhood was detected in maternal obesity or overweight by self-report (adjusted OR: 1.54 and 1.21, 95% CI: 1.34-1.77 and 1.12-1.31, both $p < 0.001$)

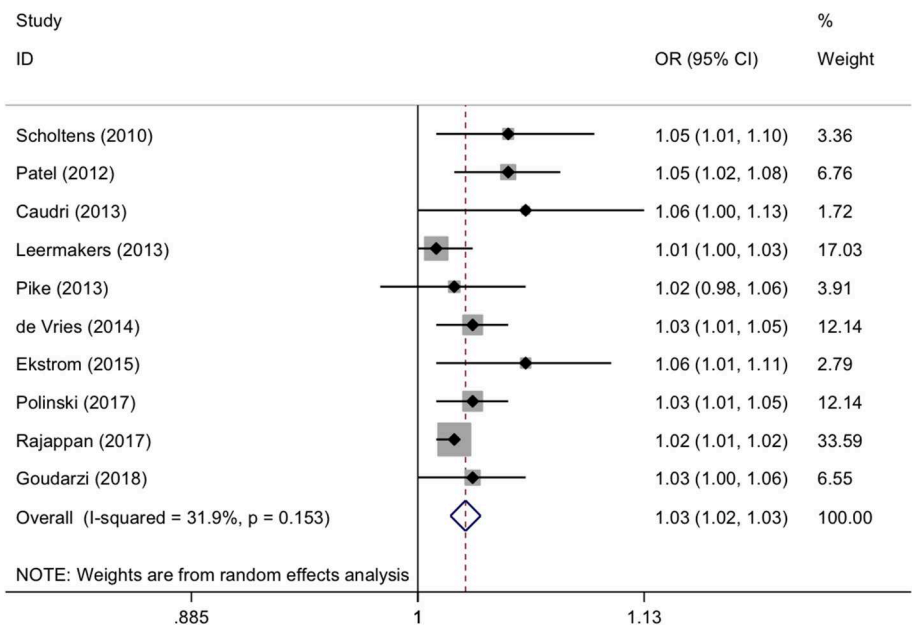

FIGURE 3 | Forest plots for continuous body mass index after adjustment. OR, odds ratio; 95% CI, 95% confidence interval. The gray shadow size represents the proportion of the weight. The black line equal to 1 perpendicular to the horizontal axis represents an invalid line, and the red dashed line parallel to the black line represents the combined effect line of all the included studies.

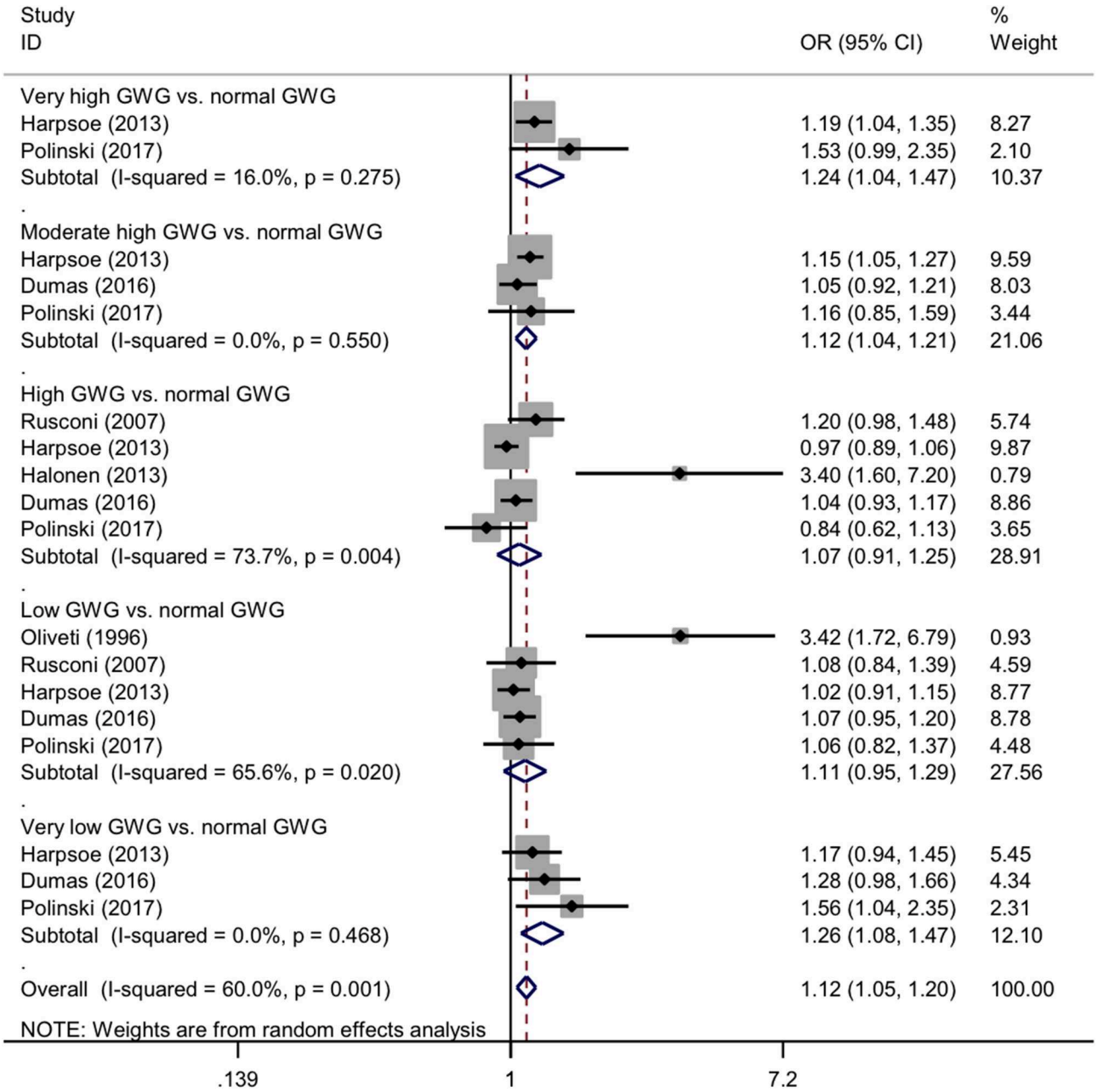

FIGURE 4 | Forest plots for categorical gestational weight gain after adjustment. GWG, gestational weight gain; OR, odds ratio; 95% CI, 95% confidence interval. The gray shadow size represents the proportion of the weight. The black line equal to 1 perpendicular to the horizontal axis represents an invalid line, and the red dashed line parallel to the black line represents the combined effect line of all the included studies.

than by Medical records (adjusted OR: 1.40 and 1.14, 95% CI: 1.12-1.74 and 0.90-1.45, *p*: 0.003 and 0.282) (**Table 2**). By region, there was no obvious difference for the association between maternal pre-pregnancy obesity or overweight and the risk of childhood asthma (**Table 2**).

Meta-Regression Analyses

To explore the extent to which study-level characteristics explained heterogeneity, meta-regression analyses were conducted by modeling averaged age and gender composition (**Supplementary Figures 4**, **5** for maternal categorical BMI in pre-pregnancy, **Supplementary Figure 6** for maternal continuous BMI in pre-pregnancy). With the increase of male percentage, the risk of childhood wheeze associated with pre-pregnancy maternal obesity was significantly decreased before adjustment (*p*: 0.017), while no significance was reached after adjustment (*p*: 0.149). With the increase of averaged age, there was marginally statistical significance

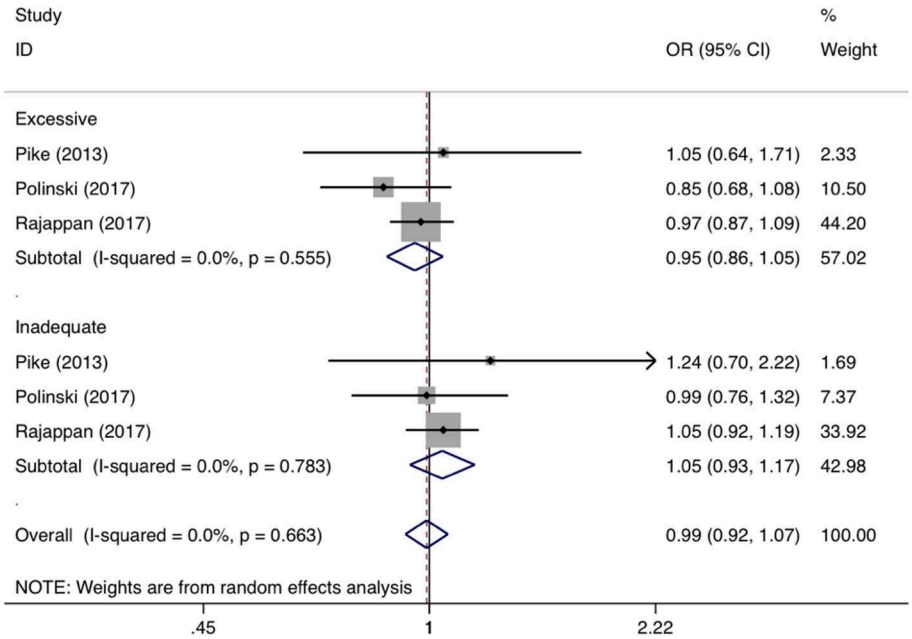

FIGURE 5 | Forest plots for categorical gestational weight gain according to Institute of Medicine criteria after adjustment. OR, odds ratio; 95% CI, 95% confidence interval. The gray shadow size represents the proportion of the weight. The black line equal to 1 perpendicular to the horizontal axis represents an invalid line, and the red dashed line parallel to the black line represents the combined effect line of all the included studies.

in the increased risk of childhood wheeze associated with pre-pregnancy maternal overweight before adjustment (*p*: 0.049), and there was no detectable significance after adjustment (*p*: 0.462).

Publication Bias

The Begg's and filled funnel plots before and after adjustment are shown in **Supplementary Figures 7**, **8**, respectively. Before adjustment, Begg's funnel plots seemed symmetrical for maternal continuous BMI, yet unsymmetrical for maternal categorical BMI and GWG, as confirmed by Egger's tests (*p*: 0.238, 0.003, and 0. 003, respectively). After adjustment, Begg's funnel plots seemed unsymmetrical in maternal BMI and GWG, as confirmed by Egger's tests (*p*: 0.011, 0.024, and 0. 003, respectively). As revealed by filled funnel plots, there were 3 to 12 estimated missing studies in the analysis of maternal BMI and GWG. Using the Duval and Tweedie "trim and fill" method to account for potential missing trials, significance was retained for unadjusted and adjusted maternal categorical BMI (OR: 1.14 and 1.121; 95% CI: 1.05-1.24 and 1.05-1.20; *p*: 0.002 and 0.001), as well as for unadjusted and adjusted maternal continuous BMI (OR: 1.02 and 1.02; 95% CI: 1.02-1.03 and 1.02-1.03; both $p < 0.001$), whereas no significance was seen for unadjusted and adjusted GWG (OR: 1.11 and 1.07; 95% CI: 1.00-1.23 and 0.99-1.16; *p*: 0.06 and 0.085). Additionally, we provided the scatter plots of sample size and publication year with effect size in **Supplementary Figures 9**, **10**, respectively.

DISCUSSION

Via a comprehensive analysis of 145,574 mother-child pairs form 22 observational studies, our results support the hypothesis that both pre-pregnancy maternal obesity or overweight and very to moderate high or low GWG left their offspring susceptible to a significantly increased risk of having childhood asthma and wheeze. Moreover, as revealed by our subgroup analyses, sample size, weight modality, and diagnosis of asthma were potential sources of between-study heterogeneity. To the best of our knowledge, this is thus far the most comprehensive meta-analysis that has evaluated the association of pre-pregnancy maternal BMI and GWG with the risk of childhood asthma and wheeze.

Differing from the results of the previous meta-analysis by Forno and colleagues in 2014 (9), asthma and wheeze were analyzed both jointly and independently due to sufficient number of eligible studies in this present meta-analysis, and prespecified subgrouping of studies was not done according to ever and current asthma or wheeze due to ambiguous and inconsistent definitions across studies. Our results confirmed previously reported association of pre-pregnancy maternal obesity or overweight with an increased risk of having childhood asthma and wheeze in offspring found by Forno and colleagues (9). Besides, as an extension of previous studies, we observed that both very to moderate high and low GWG can significantly increase the risk of childhood asthma and wheeze. Our findings are biologically plausible. On one hand, obesity is established as a chronic and low-grade inflammatory state (35). There is evidence that obese women had higher serum proinflammatory markers than women with normal weight during pregnancy

TABLE 2 | Subgroup analysis for asthma/wheeze base on categorical BMI (adjusted).

Group	Numbers (Asthma/Wheeze)	Asthma		Wheeze	
		OR (95% CI); *P*	I^2	OR (95% CI); *P*	I^2
Maternal obesity					
By sample size					
Total sample size <6000	3/7	1.60 (1.20–2.13); 0.001	48.6%	1.68 (1.25–2.26); 0.001	73.0%
Total sample size ≥6000	3/6	1.50 (1.34–1.66); <0.001	0.0%	1.28 (1.10–149); 0.001	44.9%
By region					
America	3/2	1.39 (1.20–1.60); <0.001	0.0%	3.12 (1.74–5.13); <0.001	0.0%
Asia	NA/1	NA	NA	1.67 (0.73–3.81); 0.223	NA
Europe	3/10	1.63 (1.38–1.94); <0.001	19.4%	1.29 (1.14–1.45); <0.001	56.2%
By weight modality					
Medical records	2/4	1.40 (1.12–1.74); 0.003	0.0%	1.24 (1.00–1.55); 0.052	32.7%
Self-report	4/9	1.54 (1.34–1.77); <0.001	32.3%	1.48 (1.22–1.79); <0.001	70.4%
By diagnosis of asthma					
Doctor diagnosis	5/5	1.50 (1.33–1.69); <0.001	23.9%	1.48 (1.19–1.86); 0.001	36.1%
Parental report	1/8	1.53 (1.04–2.26); 0.032	NA	1.29 (1.11–1.51); 0.001	63.0%
Maternal overweight					
By sample size					
Total sample size <6000	4/8	1.19 (0.95–1.49); 0.123	47.1%	1.19 (0.95–1.49); 0.032	0.0%
Total sample size ≥6000	3/5	1.21 (1.13–1.30); <0.001	0.0%	1.21 (1.13–1.30); 0.022	41.9%
By region					
America	2/1	1.20 (1.06–1.38); 0.005	0.0%	1.58 (0.75–3.31); 0.226	NA
Asia	NA/1	NA	NA	1.11 (0.72–1.71); 0.635	NA
Europe	5/11	1.21 (1.06–1.37); 0.004	30.9%	1.07 (1.02–1.11); 0.003	0.0%
By weight modality					
Medical records	1/3	1.14 (0.90–1.45); 0.282	NA	1.12 (1.03–1.23); 0.013	0.0%
Self-report	6/10	1.21 (1.12–1.31); <0.001	10.6%	1.06 (1.01–1.12); 0.028	2.7%
By diagnosis of asthma					
Doctor diagnosis	4/4	1.22 (1.14–1.31); <0.001	0.0%	1.16 (1.03–1.30); 0.013	0.0%
Parental report	3/9	1.14 (0.86–1.51); 0.362	57.9%	1.06 (1.01–1.10); 0.019	0.0%

BMI, body mass index; OR, odds ratio; 95% CI, 95% confidence interval; I^2, inconsistency index; NA, not available.

(36), and elevated inflammatory markers during pregnancy were closely linked to wheeze in offspring (37). Pregnancy per se is characterized by immunomodulatory changes (38), which along with obesity-driven proinflammatory state might affect fetal immune system development by placenta, and thus predispose the offspring to childhood asthma and wheeze (39, 40). On the other hand, diverse dietary patterns may contribute to the relation between maternal obesity and childhood asthma. For instance, maternal high-fat intake during pregnancy or meat intake before pregnancy was associated with an elevated risk of childhood asthma or wheeze (41, 42). In support of this association, experiment studies showed that asthma was a developmental origin disease influenced by maternal diets (43). Although a large number of studies have been conducted to explore and explain the association between maternal obesity and offspring asthma and wheeze, the exact mechanism of action underlying this association needs to be further elucidated. Nonetheless, from a public health viewpoint, our meta-analytical findings underscore the importance of mastering maternal weight gain in pre-pregnancy and during gestation and controlling them within a reasonable range to lower the future risk of suffering childhood asthma and wheeze in offspring.

It is worth noting that there was a dose-dependent relation between pre-pregnancy maternal weight and the risk for asthma and wheeze in childhood. In this meta-analysis, per pre-pregnancy BMI increment after adjustment was associated with an 3% increased risk of childhood asthma and wheeze, which was consistent with the result by Forno et al. (9). In support of this dose-dependent relation, adjusted pooled odds for childhood asthma and wheeze was increased from 1.13 for maternal overweight to 1.41 for maternal obesity in pre-pregnancy. Although the possibility of residual confounding cannot be fully eliminated, our findings seem reliable and robust, as effect-size estimates are still significant even after accounting for potentially missing studies as revealed by the trim-and-fill method.

Extending the findings of previous meta-analysis (9), we employed both subgroup and meta-regression analyses to seek

the reasons for previously inconsistent results, and interestingly we found that sample size, weight modality, and diagnosis of asthma were potential sources of between-study heterogeneity. In particular, the association between pre-pregnancy maternal obesity or overweight and increased asthma and wheeze risk in this meta-analysis was more obvious in small studies than large studies, as well as in studies with doctor-diagnosed asthma than studies with parent-reported asthma, and such association was epidemiologically plausible. The significance in studies with large sample sizes reinforced the robustness of our principal finding. As for the diagnosis of asthma, a cross-sectional and longitudinal epidemiological survey revealed that the prevalence of asthma was 15.5% according to parental reports, and 21.5% according to doctor's diagnosis (44), indicating that asthma reported by parents may underestimate the risk of childhood asthma and wheeze related to asthma diagnosed by doctors. Contrastingly, there was no detectable significance in meta-regression analysis, likely due to its lack of the methodological rigor (45). It is expected that analysis of individual participants' data could make up methodological drawbacks and yield further insights, which is not practically feasible.

Despite the clear strengths of this meta-analysis, including large sample sizes, careful assessment of maternal weight, and comprehensive explorations on between-study heterogeneity, some possible limitations should be acknowledged when interpreting our findings. Firstly, only five pubic databases were reviewed for literature search, and this meta-analysis merely focused on articles published in the English language, which might yield a selection bias. Additionally, a majority of involved studies were longitudinal in design and with varied follow-up intervals, which may not capture the event of interest especially for studies with short follow-ups. Secondly, analysis on categorical GWG was based on only six studies, which precluded further subgroup analyses. Thirdly, in this meta-analysis, the categorization of asthma, such as ever, current, and persistent asthma was lack. Fourthly, some, but not all included studies had adjusted for child's weight or BMI at the time of assessment, indicating that the effect of maternal obesity on asthma and wheeze is not independent of childhood obesity, which is an established risk factor for asthma and wheeze (46).

Taken together, our findings indicate that both pre-pregnancy maternal obesity or overweight and very to moderate high or low GWG left their offspring susceptible to a significantly increased risk of having childhood asthma and wheeze. Moreover, sample size and diagnosis of asthma were potential sources of between-study heterogeneity. These data suggest that maintenance of maternal weight gain in pre-pregnancy and during gestation needs to be implemented as a primary prevention of the future development of childhood asthma and wheeze in offspring.

DATA AVAILABILITY STATEMENT

The datasets analyzed in this article are not publicly available. Requests to access the datasets should be directed to zhangzhixin032@163.com.

AUTHOR CONTRIBUTIONS

ZZ and WN conceived and designed the experiments. SL and BZ performed the experiments. SL and WN analyzed the data and wrote the paper. SL, YW, KW, and BZ contributed materials/analysis tools. All authors read and approved the final manuscript prior to submission.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2020.00134/full#supplementary-material

REFERENCES

1. Serebrisky D, Wiznia A. Pediatric asthma: a Global epidemic. *Ann Glob Health.* (2019) 85:2416. doi: 10.5334/aogh.2416
2. Loftus PA, Wise SK. Epidemiology of asthma. *Cur Opin Otolaryngol Head Neck Surg.* (2016) 24:245–9. doi: 10.1097/MOO.0000000000000262
3. Castro-Rodriguez JA, Forno E, Rodriguez-Martinez CE, Celedon JC. Risk and protective factors for childhood asthma: what is the evidence? *J Allergy Clin Immunol Prac.* (2016) 4:1111–22. doi: 10.1016/j.jaip.2016.05.003
4. Dumas O, Varraso R, Gillman MW, Field AE, Camargo CA. Longitudinal study of maternal body mass index, gestational weight gain, and offspring asthma. *Allergy.* (2016) 71:1295–304. doi: 10.1111/all.12876
5. Kim SY, Dietz PM, England L, Morrow B, Callaghan WM. Trends in pre-pregnancy obesity in nine states, 1993-2003. *Obesity.* (2007) 15:986–93. doi: 10.1038/oby.2007.621
6. Godfrey KM, Reynolds RM, Prescott SL, Nyirenda M, Jaddoe VW, Eriksson JG, et al. Influence of maternal obesity on the long-term health of offspring. *Lancet Diabetes Endocrinol.* (2017) 5:53–64. doi: 10.1016/S2213-8587(16)30107-3
7. Polinski KJ, Liu J, Boghossian NS, McLain AC. Maternal obesity, gestational weight gain, and asthma in offspring. *Prevent Chronic Dis.* (2017) 14:E109. doi: 10.5888/pcd14.170196
8. Harpsoe MC, Basit S, Bager P, Wohlfahrt J, Benn CS, Nohr EA, et al. Maternal obesity, gestational weight gain, and risk of asthma and atopic disease in offspring: a study within the danish national birth cohort. *J Allergy Clin Immunol.* (2013) 131:1033–40. doi: 10.1016/j.jaci.2012.09.008
9. Forno E, Young OM, Kumar R, Simhan H, Celedon JC. Maternal obesity in pregnancy, gestational weight gain, and risk of childhood asthma. *Pediatrics.* (2014) 134:e535–46. doi: 10.1542/peds.2014-0439
10. de Vries A, Reynolds RM, Seckl JR, van der Wal M, Bonsel GJ, Vrijkotte TG. Increased maternal BMI is associated with infant wheezing in early life: a prospective cohort study. *J Dev Origins Health Dis.* (2014) 5:351–60. doi: 10.1017/S2040174414000312
11. Ekstrom S, Magnusson J, Kull I, Lind T, Almqvist C, Melen E, et al. Maternal body mass index in early pregnancy and offspring asthma, rhinitis and eczema up to 16 years of age. *Clin Exp Allergy.* (2015) 45:283–91. doi: 10.1111/cea.12340
12. Harskamp-van Ginkel MW, London SJ, Magnus MC, Gademan MG, Vrijkotte TG. A study on mediation by offspring BMI in the association between maternal obesity and child respiratory outcomes in the amsterdam born and their development study cohort. *PLoS ONE.* (2015) 10:e0140641. doi: 10.1371/journal.pone.0140641
13. Taylor-Robinson DC, Pearce A, Whitehead M, Smyth R, Law C. Social inequalities in wheezing in children: findings from the UK millennium

cohort study. *Eur Resp J.* (2016) 47:818–28. doi: 10.1183/13993003.01117-2015
14. Rajappan A, Pearce A, Inskip HM, Baird J, Crozier SR, Cooper C, et al. Maternal body mass index: relation with infant respiratory symptoms and infections. *Pediatric Pulmonol.* (2017) 52:1291–9. doi: 10.1002/ppul.23779
15. Goudarzi H, Konno S, Kimura H, Araki A, Miyashita C, Itoh S, et al. Contrasting associations of maternal smoking and pre-pregnancy bMI with wheeze and eczema in children. *Sci Total Environ.* (2018) 639:1601–9. doi: 10.1016/j.scitotenv.2018.05.152
16. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med.* (2009) 6:e1000097. doi: 10.1371/journal.pmed.1000097
17. Kim RS. A new comparison of nested case-control and case-cohort designs and methods. *Eur J Epidemiol.* (2015) 30:197–207. doi: 10.1007/s10654-014-9974-4
18. Stang A. Critical evaluation of the newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. *Eur J Epidemiol.* (2010) 25:603–5. doi: 10.1007/s10654-010-9491-z
19. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ.* (2003) 327:557–60. doi: 10.1136/bmj.327.7414.557
20. Lin L, Chu H. Quantifying publication bias in meta-analysis. *Biometrics.* (2018) 74:785–94. doi: 10.1111/biom.12817
21. Shi L, Lin L. The trim-and-fill method for publication bias: practical guidelines and recommendations based on a large database of meta-analyses. *Medicine.* (2019) 98:e15987. doi: 10.1097/MD.0000000000015987
22. Oliveti JF, Kercsmar CM, Redline S. Pre- and perinatal risk factors for asthma in inner city African-American children. *Am J Epidemiol.* (1996) 143:570–7. doi: 10.1093/oxfordjournals.aje.a008787
23. Rusconi F, Galassi C, Forastiere F, Bellasio M, De Sario M, Ciccone G, et al. Maternal complications and procedures in pregnancy and at birth and wheezing phenotypes in children. *Am J Resp Critical Care Med.* (2007) 175:16–21. doi: 10.1164/rccm.200512-1978OC
24. Reichman NE, Nepomnyaschy L. Maternal pre-pregnancy obesity and diagnosis of asthma in offspring at age 3 years. *Maternal Child Health J.* (2008) 12:725–33. doi: 10.1007/s10995-007-0292-2
25. Haberg SE, Stigum H, London SJ, Nystad W, Nafstad P. Maternal obesity in pregnancy and respiratory health in early childhood. *Paediat Perinatal Epidemiol.* (2009) 23:352–62. doi: 10.1111/j.1365-3016.2009.01034.x
26. Kumar R, Story RE, Pongracic JA, Hong X, Arguelles L, Wang G, et al. Maternal pre-pregnancy obesity and recurrent wheezing in early childhood. *Pediat Allergy Immunol Pulmonol.* (2010) 23:183–90. doi: 10.1089/ped.2010.0032
27. Scholtens S, Wijga AH, Brunekreef B, Kerkhof M, Postma DS, Oldenwening M, et al. Maternal overweight before pregnancy and asthma in offspring followed for 8 years. *Int J Obesity.* (2005) 34:606–13. doi: 10.1038/ijo.2009.194
28. Patel SP, Rodriguez A, Little MP, Elliott P, Pekkanen J, Hartikainen AL, et al. Associations between pre-pregnancy obesity and asthma symptoms in adolescents. *J Epidemiol Commun Health.* (2012) 66:809–14. doi: 10.1136/jech.2011.133777
29. Caudri D, Savenije OE, Smit HA, Postma DS, Koppelman GH, Wijga AH, et al. Perinatal risk factors for wheezing phenotypes in the first 8 years of life. *Clin Exp Allergy.* (2013) 43:1395–405. doi: 10.1111/cea.12173
30. Guerra S, Sartini C, Mendez M, Morales E, Guxens M, Basterrechea M, et al. Maternal prepregnancy obesity is an independent risk factor for frequent wheezing in infants by age 14 months. *Paediat Perin Epidemiol.* (2013) 27:100–8. doi: 10.1111/ppe.12013
31. Halonen M, Lohman IC, Stern DA, Ellis WL, Rothers J, Wright AL. Perinatal tumor necrosis factor-alpha production, influenced by maternal pregnancy weight gain, predicts childhood asthma. *Am J Resp Critical Care Med.* (2013) 188:35–41. doi: 10.1164/rccm.201207-1265OC
32. Leermakers ET, Sonnenschein-van der Voort AM, Gaillard R, Hofman A, de Jongste JC, Jaddoe VW, et al. Maternal weight, gestational weight gain and preschool wheezing: the Generation R Study. *Eur Resp J.* (2013) 42:1234–43. doi: 10.1183/09031936.00148212
33. Pike KC, Inskip HM, Robinson SM, Cooper C, Godfrey KM, Roberts G, et al. The relationship between maternal adiposity and infant weight gain, and childhood wheeze and atopy. *Thorax.* (2013) 68:372–9. doi: 10.1136/thoraxjnl-2012-202556
34. Wright RJ, Fisher K, Chiu YH, Wright RO, Fein R, Cohen S, et al. Disrupted prenatal maternal cortisol, maternal obesity, and childhood wheeze. Insights into prenatal programming. *Am J Resp Crit Care Med.* (2013) 187:1186–93. doi: 10.1164/rccm.201208-1530OC
35. Cancello R, Clement K. Is obesity an inflammatory illness? Role of low-grade inflammation and macrophage infiltration in human white adipose tissue. *BJOG.* (2006) 113:1141–7. doi: 10.1111/j.1471-0528.2006.01004.x
36. Christian LM, Porter K. Longitudinal changes in serum proinflammatory markers across pregnancy and postpartum: effects of maternal body mass index. *Cytokine.* (2014) 70:134–40. doi: 10.1016/j.cyto.2014.06.018
37. Morales E, Guerra S, Garcia-Esteban R, Guxens M, Alvarez-Pedrerol M, Bustamante M, et al. Maternal c-reactive protein levels in pregnancy are associated with wheezing and lower respiratory tract infections in the offspring. *Am J Obstet Gynecol.* (2011) 204:164 e1–9. doi: 10.1016/j.ajog.2010.08.056
38. Arck PC, Hecher K. Fetomaternal immune cross-talk and its consequences for maternal and offspring's health. *Nat Med.* (2013) 19:548–56. doi: 10.1038/nm.3160
39. Rusconi F, Popovic M. Maternal obesity and childhood wheezing and asthma. *Paediat Resp Rev.* (2017) 22:66–71. doi: 10.1016/j.prrv.2016.08.009
40. Rizzo GS, Sen S. Maternal obesity and immune dysregulation in mother and infant: a review of the evidence. *Paediat Resp Rev.* (2015) 16:251–7. doi: 10.1016/j.prrv.2014.10.005
41. Fitzsimon N, Fallon U, O'Mahony D, Loftus BG, Bury G, Murphy AW, et al. Mothers' dietary patterns during pregnancy and risk of asthma symptoms in children at 3 years. *Irish Med J.* (2007) 100:suppl 27–32.
42. Baiz N, Just J, Chastang J, Forhan A, de Lauzon-Guillain B, Magnier AM, et al. Maternal diet before and during pregnancy and risk of asthma and allergic rhinitis in children. *Allergy Asthma Clin Immunol.* (2019) 15:40. doi: 10.1186/s13223-019-0353-2
43. Thorburn AN, McKenzie CI, Shen S, Stanley D, Macia L, Mason LJ, et al. Evidence that asthma is a developmental origin disease influenced by maternal diet and bacterial metabolites. *Nat Commun.* (2015) 6:7320. doi: 10.1038/ncomms8320
44. Canova C, Harris JM, Mills P, White C, Moffat S, Shread L, et al. Epidemiological measures of childhood asthma: cross-sectional and longitudinal consistency. *Respir Med.* (2012) 106:1226–35. doi: 10.1016/j.rmed.2012.05.008
45. Munafo MR, Flint J. Meta-analysis of genetic association studies. *Trends Genet.* (2004) 20:439–44. doi: 10.1016/j.tig.2004.06.014
46. Deng X, Ma J, Yuan Y, Zhang Z, Niu W. Association between overweight or obesity and the risk for childhood asthma and wheeze: an updated meta-analysis on 18 articles and 73 252 children. *Pediat Obesity.* (2019) 14:e12532. doi: 10.1111/ijpo.12532

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Perinatal Determinants of Child Maltreatment in Japan

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Background: Child maltreatment induces significant health problems, both during childhood and into adulthood. To prevent child maltreatment, it is important to detect perinatal risk factors for earlier intervention. The aim of this study was to evaluate the perinatal risk factors associated with child maltreatment during pregnancy.

Methods: A case-control study was conducted to compare perinatal data from the Maternal and Child Health Handbook between the case and control groups. Cases were collected from children registered in two Child Guidance Centers in Japan. The control group consisted of 3.5-year-old children in a city in Osaka Prefecture whose mothers responded to questionnaires containing information from the Maternal and Child Health Handbook. The association between perinatal factors and child maltreatment was assessed using multiple logistic regression analysis.

Results: The data of 70 cases and 345 controls were collected. The following were found to be perinatal factors related to child maltreatment: teenage pregnancy (OR: 257.3, 95% CI: 17.3–3832.7), a mother aged 20–24 years (OR: 22.8, 95% CI: 4.4–117.8), a father who is older than the mother by 10 years or more (OR: 14.1, 95% CI: 2.1–94.8), an unmarried mother (OR: 15.7, 95% CI: 2.6–93.6), maternal mental disorder (OR: 48.9, 95% CI: 9.3–258.3), the first maternal prenatal visit being later than 20 weeks (OR: 132, 95% CI: 12.7–1384.7), little prenatal care (<10 visits) (OR: 21.4, 95% CI: 2.9–157.1), a low-birth-weight baby (OR: 5.1, 95% CI: 1.1–24.1), and congenital disease (OR: 7.9, 95% CI: 1.1–56.4).

Conclusions: This study revealed that young mothers, fathers much older than mothers, unmarried mothers, and maternal mental disorder, mothers with late first visit or little perinatal care, and low-birth-weight babies and babies with congenital disease were associated with child maltreatment. These findings can be used to detect high-risk families for child maltreatment during or after pregnancy.

Keywords: child maltreatment, perinatal factors, the maternal and child health handbook, the child protection register, screening high-risk families during pregnancy

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INTRODUCTION

Child maltreatment causes significant problems in child development (1–3). It is a serious global problem and Japan is no exception (4). The latest data indicates that, from April 2016 to March 2017, a total of 49 deaths were due to child abuse (5). Among the deaths, children under 1 year of age accounted for 32 (65.3%), of which 16 (50.0%) were within the first month of life. These numbers suggest the importance of early prevention efforts against child maltreatment from pregnancy onward.

Maternal perinatal risk factors for child maltreatment have been reported and the recognition of families with a high risk of child maltreatment through gestation is an effective means for early prevention (6–9). This is reasonable approach for early detection of child maltreatment, which uses data that are easily measured during regular prenatal care. Therefore, in this survey, we used the Maternal and Child Health Handbook (MCH). In Japan, a woman is issued with the MCH after pregnancy is confirmed (10, 11). Japan has a pregnancy registration system, subsidies for pregnancy health checkups, and a universal healthcare system. The MCH is a comprehensive home-based health booklet for mothers and children that is distributed at local government offices. It records the health condition of the mother throughout pregnancy, delivery, and the postnatal period, as well as the condition of the child's development and immunization. In addition, it plays a role in health education about pregnancy, birth, neonates, childcare, nutrition, dental care, and immunization. Medical records are written down in the handbook by obstetricians, pediatricians, midwives, and public health nurses at hospitals, clinics, or health centers. Parents bring the handbook to hospitals or clinics when their children receive a routine vaccination or fall sick. Pregnant women receive regular (at least 14) prenatal checkups at obstetric facilities. In a prenatal checkup, the mother's health condition is assessed and a medical examination and health guidance are conducted. A newborn receives a medical examination at an obstetric facility at one month old with the mother, and subsequently, an infant medical examination is conducted in municipal health centers at the age of 3 months, 1.5 years, and 3.5 years (12, 13). In Japan, the reason for placing children on the child protection register is not only child maltreatment but insufficient nurturing, such as when their mother has a disease. We examined the perinatal risk factors related with child maltreatment listed in the MCH, by comparing children who had been maltreated and registered at a Child Guidance Center with children who had never been maltreated up until their 3.5-year health examination.

MATERIALS AND METHODS

This was an unmatched case-control study comparing perinatal data from the MCH between children in foster care and those who had never been registered for child maltreatment.

In this study, the cases comprised 0- to 5-year-old children who had been placed on the child protection register for child maltreatment at two Child Guidance Centers in Osaka Prefecture. The cases were selected from the entire area of Osaka Prefecture and from the surrounding prefecture. We limited the sample to mothers with their copies of the MCH. We asked health nurses on the staff of the two Child Guidance Centers to provide information about the children placed on the child protection register. We received information regarding their MCH from the nurses with the exception of personal information such as name, address, and birth date. We could not obtain the consent from parents of the cases because the cases had been placed on the child protection register for child maltreatment. Instead, the cases survey was conducted with the consent of the Child Guidance Centers and Osaka Prefecture. The control group consisted of 3.5-year-old children living in a city in Osaka Prefecture whose mothers had agreed to be the subjects of this study at the infant health examination. We obtained the written informed consent from the parents of participants. The mothers answered a questionnaire, including information regarding the MCH. On the questionnaire a total of 27 question items were set, and closed or multiple-choice question was prepared for the respective items with the exception of maternal and paternal age, gestational age, the week of the first visit, number of pregnancy medical examinations and birth weight. The public health nurses in the city confirmed the information given in the questionnaires, and if there were any blank responses, they asked the mothers to complete them. This questionnaire survey was conducted among all mothers who have 3.5-year-old children living in the city. As a result, the survey was also conducted with mothers of children requiring the city's care because of maltreatment. In Japan, the most severely affected children are placed on the child protection register and less severely affected ones are living with their parents and are watched under the city's care. However we excluded them from the control group. In addition, we also excluded children who had no detailed information in their MCH. The sample size was calculated using the assumption of 95% confidence interval, and 80% power with one to five ratio of cases and controls using the hypothetical proportion of 5% with perinatal risk in controls, to detect an odds ratio of 3.0 or greater.

We examined the following perinatal factors: background factors, gestational factors, and children's factors. The background factors consisted of maternal age, paternal age, a much older father, number of children (including the subjects of this study), economic status, partner status, and maternal mental disorder. Economic status was defined as whether welfare or public assistance benefits had been received for the cost of delivery. Among the cases, maternal mental disorder included mothers who were considered to have psychiatric problems by the foster home staff, as well as those who were diagnosed with a mental disorder. In the control group, information about maternal mental disorder was composed of self-reported data from the questionnaire. In the questionnaire, it was included as one of the options to obtain the medical history. The gestational factors consisted of the week of the first visit, number of pregnancy medical examinations, hypertensive disorder of pregnancy, proteinuria, blood transfusion, and method of delivery, which were obtained from the MCH. The children's factors consisted of multiple pregnancy,

Abbreviations: MCH, the Maternal and Child Health Handbook.

congenital disease of children, birth weight, and gestational age at delivery.

We compared continuous variables using the Wilcoxon rank-sum test because the variables did not have a normal distribution, and categorical variables using Pearson's chi-square and Fisher's exact tests. The association between maternal mental disorder or maternal age and other factors was examined by chi-square test with cross tabulation. Perinatal factors related to child maltreatment were evaluated using multiple logistic regression analysis after adjusting the age of admission to the child protection register or the age at the time of investigation. The Variance Inflation Factor (VIF) was calculated using the regression analysis to confirm multi-collinearity in the multivariate analysis. There were some significant variables in the univariate analysis that were excluded in the multivariate analysis. Twin pregnancy was not included in the multivariate analysis because there were only 10 pairs across both groups. Because proteinuria and hypertensive disorder of pregnancy, maternal and paternal age, and premature birth and cesarean section are closely related, we selected only hypertensive disorder of pregnancy, maternal age, and preterm birth as factors, respectively, for the multivariate analysis. The week of the first visit and number of pregnancy medical examinations were correlated. Therefore, the factor of the week of the first visit being before 20 weeks' gestation and with more than 10 examinations was used as a reference for two situations: the first visit being after 20 weeks' gestation, regardless of the number of examinations, and the first visit being before 20 weeks' gestation with less than 10 examinations. Statistical analyses were performed using Stata software, version 14.0 (Stata). Two-sided p-values of less than 0.05 were considered to indicate statistical significance.

RESULTS

Between April 2013 and March 2016, there were 97 children aged 0 to 5 years who had been placed on the child protection register at the two Child Guidance Centers in Osaka Prefecture and who had their copies of the MCH. A total of 27 children were excluded because they had been placed on the child protection register because of insufficient nurturing, not child maltreatment. The 70 remaining children had been placed on the child protection register because of child maltreatment. Among the causes, physical abuse accounted for 33 (47.1%); neglect, 47 (67.1%); and emotional abuse, 17 (24.3%). The type of abuse was duplicated in some cases (**Figure 1**).

From May 2015 to April 2016, 576 children aged 3.5 years underwent the infant health examination at a city in Osaka Prefecture. There were 370 (64.2%) of their mothers who agreed and responded to the survey. A total of 25 cases were excluded either because of lack of detailed information ($n = 19$) or because of children requiring aid due to maltreatment ($n = 6$). Thus, 345 cases were included in the analysis. **Table 1** shows the comparison of background factors, gestational factors, and children's factors in the registered and control groups. There was a significant difference between the registered and control groups in almost all the factors. The proportion of young parents was significantly higher in the cases than in the control group.

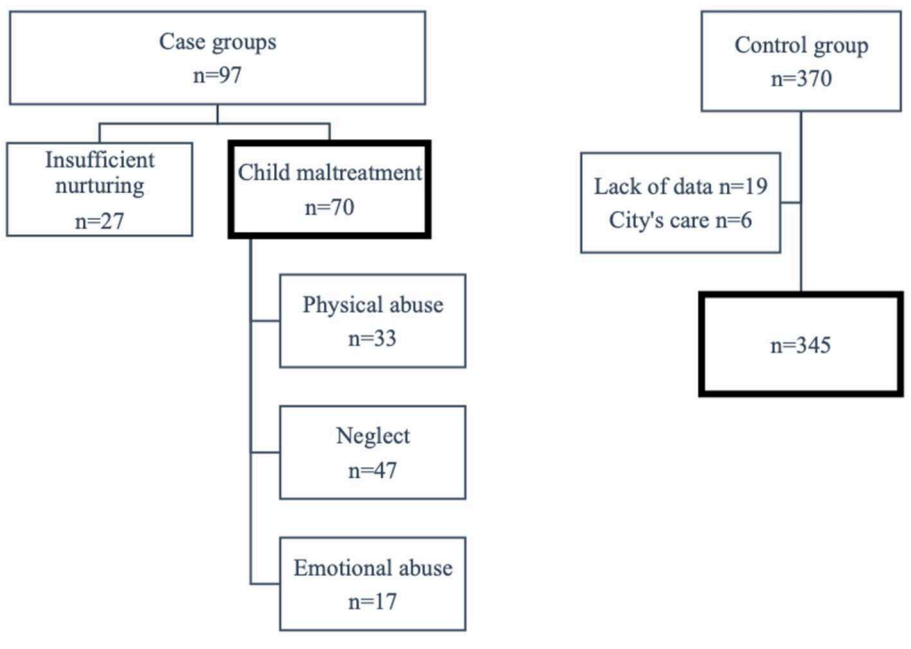

FIGURE 1 | The flowchart of study population. A total of 27 children were excluded because they had been placed on the child protection register because of insufficient nurturing, not child maltreatment. Among the causes, physical abuse accounted for 33 (47.1%); neglect, 47 (67.1%); and emotional abuse, 17 (24.3%). The type of abuse was duplicated in some cases. A total of 25 cases were excluded either because of lack of detailed information ($n = 19$) or because of children requiring aid due to maltreatment ($n = 6$).

TABLE 1 | Comparison of background factors, gestational factors, and children's factors in the case and control groups.

Risk factors	Case group ($n = 70$)	Control group ($n = 345$)	*P*
BACKGROUND FACTORS			
Maternal age	23 (14–40)	31 (17–43)	<0.001
Teenage pregnancy	14/70 (20.0%)	3/345 (0.9%)	<0.001
Paternal age	29 (14–72)	33 (19–54)	0.002
Father over 10 years older than the mother	12/58 (20.7%)	18/338 (5.3%)	<0.001
Number of children ≥3	29/70 (41.4%)	69/345 (20%)	<0.001
Economic poverty	33/68 (48.5%)	28/343 (8.2%)	<0.001
Unmarried mother	35/70 (50%)	10/345 (2.9%)	<0.001
Maternal mental disorder	33/70 (47.1%)	13/345 (3.8%)	<0.001
GESTATIONAL FACTORS			
Gestational age at the first visit	14 (7–40)	9 (4–38)	<0.001
First visit ≥20 wks[a]	16/66 (24.2%)	2/324 (0.6%)	<0.001
Number of pregnancy medical examinations	10 (0–15)	13 (7–20)	<0.001
First visit <20 wks[a] and number of examinations <10	17/66 (25.8%)	8/319 (2.5%)	<0.001
Hypertensive disorder of pregnancy	8/66 (12.1%)	5/341 (1.5%)	<0.001
Proteinuria	44/66 (66.7%)	119/341 (34.9%)	<0.001
Blood transfusion	1/54 (1.9%)	4/343 (1.2%)	0.67
Cesarean section	29/70 (41.4%)	56/343 (16.3%)	<0.001
Gestational age of delivery	38 (28–41)	39 (33–42)	<0.001
Preterm birth	18/69 (26.1%)	11/345 (3.2%)	<0.001
CHILDREN'S FACTORS			
Twins	6/70 (8.6%)	10/345 (2.9%)	0.025
Children's age at registration or investigation	2.5 (0–5)	3.5	
Congenital disease	8/69 (11.6%)	10/345 (2.9%)	0.001
Birth weight (gms)	2723 (828–4002)	3034 (1222–4182)	<0.001
Low birth weight	23/70 (32.9%)	28/343 (8.2%)	<0.001

[a]*wks - weeks' gestation.*

There was a significant difference in the registered and control groups in almost all of the factors. Data are given as median (range) or n (%).

In addition, cases in which the father was more than 10 years older than the mother comprised 20.7% of the registered group, which was significantly higher than in the control group (5.3%). The proportion of unmarried parents among the cases was 50%, which was significantly higher compared to the control group (2.9%), and 82.3% of those were teenagers. Further, 48.5% of the cases had received financial assistance for helping with birth costs, which was significantly higher compared to the control group (8.2%; $p < 0.001$). Among the cases there were 33(47.1%) mothers with mental disorder, 26 of whom (78.8%) were diagnosed by a doctor. This rate was significantly higher than that in the control group (3.8%). A statistically significant difference was seen between the groups with regard to gestational age at the first visit and number of pregnancy medical examinations. Among the cases, five mothers had delivered a baby without a pregnancy examination. Hypertensive disorder of pregnancy, proteinuria, cesarean section, and preterm birth were all significantly higher in the cases than in the control group. The number of preterm births and low-birth-weight infants was significantly higher in the cases than in the control group. The rate of congenital disease among children was 11.6% among the cases, which was significantly higher than that in the control group (2.9%). All the twins among the cases were preterm births, whereas all the twins in the control group were term births.

The results of the multivariable logistic regression analysis for perinatal factors associated with child maltreatment are presented in **Table 2**. The analysis was adjusted for the age of admission to the child protection register or the age at the time of investigation. In cross tabulation, maternal mental disorder was associated with younger mother, father over 10 years older than the mother, an unmarried mother, poverty, the first maternal prenatal visit being later than 20 weeks, little prenatal care, prolificacy, hypertensive disorder of pregnancy, preterm birth, and low-birth-weight baby. Moreover, teenager was associated with father over 10 years older than the mother, an unmarried mother, poverty, the first maternal prenatal visit being later than 20 weeks, little prenatal care, and prolificacy. However, the VIF of the final model was 1.29, which was small enough to conclude this analysis was still valid. As perinatal factors related to child maltreatment, we observed the following: teenage pregnancy (OR; 257.3, 95% CI;17.3 to 3832.7), a mother aged 20–24 years (OR; 22.8, 95% CI;4.4 to 117.8), a father older by 10 years or more than the mother (OR;14.1, 95% CI;2.1 to 94.8), an unmarried mother (OR;15.7, 95% CI;2.6 to 93.6), maternal mental disorder (OR;48.9, 95% CI;9.3 to 258.3), the first maternal prenatal visit being later than 20 weeks (OR;132, 95% CI;12.7 to 1384.7), little prenatal care (less than 10 visits) (OR;21.4, 95% CI;2.9 to 157.1), a low-birth-weight baby (OR;5.1, 95% CI;1.1 to 24.1), and

TABLE 2 | Multivariable analyses for perinatal factors associated with children in foster care compared with non-abused children.

Risk factors	cOR[b] (95% confidence interval)	aOR[c] (95% confidence interval)
Maternal age	0.8 (0.8 to 0.9)	
<20	45.1 (12.3 to 165.1)	257.3 (17.3 to 3832.7)
20–24	9.7 (4.9 to 19.1)	22.8 (4.4 to 117.8)
>25	1.0	1.0
Parents' age gap (father's – mother's)		
<10	1.0	1.0
≥10	4.6 (2.1 to 10.3)	14.1 (2.1 to 94.8)
Paternal age	0.9 (0.88 to 0.97)	
Single		
Yes	33.5 (15.3 to 73.4)	15.7 (2.6 to 93.6)
No	1.0	1.0
Number of children		
1	2.2 (1.1 to 4.3)	0.2 (0.04 to 1.3)
2	1.0	1.0
3	2.8 (1.4 to 5.6)	1.0 (0.2 to 4.9)
≥4	10.1 (3.8 to 36.6)	9.3 (0.9 to 93.1)
Economic poverty		
Yes	10.6 (5.7 to 19.6)	3.6 (0.8 to 16.8)
No	1.0	1.0
Maternal mental disorder		
Yes	22.8 (11.0 to 47.1)	48.9 (9.3 to 258.3)
No	1.0	1.0
Gestational age at the first visit[d]	1.4 (1.3 to 1.5)	
Number of pregnancy medical examinations	0.5 (0.4 to 0.6)	
First visit week ≥20 wks[a]	89.0 (19.8 to 398.9)	132 (12.7 to 1384.7)
First visit week <20 wks[a] and number of examinations <10	19.9 (8.0 to 49.6)	21.4 (2.9 to 157.1)
First visit week <20 wks[a] and number of examinations ≥10	1.0	1.0
Missing data	0.4 (0.1 to 2.7)	
Hypertensive disorder of pregnancy		
Yes	9.3 (2.9 to 29.3)	2.9 (0.3 to 30.8)
No	1.0	1.0
Proteinuria[d]		
Yes	3.7 (2.1 to 6.5)	
No	1.0	
Blood transfusion		
Yes	1.6 (0.2 to 14.6)	
No	1.0	
Cesarean section		
Yes	3.6 (2.1 to 6.3)	
No	1.0	
Gestational age of delivery	0.7 (0.6 to 0.8)	
Preterm birth		
Yes	10.6 (4.7 to 23.7)	1.52 (0.2 to 15.2)
No	1.0	1.0
Twins		
Yes	3.1 (1.1 to 8.9)	
No	1.0	
Birth weight (gms)	0.99 (0.99 to 0.99)	
Low-birth-weight baby		
Yes	5.5 (2.9 to 10.3)	5.1 (1.1 to 24.1)
No	1.0	1.0
Congenital disease		
Yes	4.4 (1.7 to 11.6)	7.9 (1.1 to 56.4)
No	1.0	1.0

[a]wks - weeks' gestation; [b]crude odds ratio; [c]aOR - adjusted odds ratio.
The values were adjusted for the age of admission to the child protection register or age at the time of investigation.

congenital disease (OR;7.9, 95% CI;1.1 to 56.4). The area under receiver operating characteristic curve (AUC) was 0.971 (95% CI = 0.942 to 1.000).

DISCUSSION

We investigated the perinatal factors from the MCH associated with child maltreatment, by comparing children who had entered foster care with those who had never been registered for child maltreatment. The background factors found to be related to child maltreatment were being a mother aged between 20 and 24 years, teenage pregnancy, a father over 10 years older than the mother, an unmarried mother, and maternal mental disorder. However, age differences between parents are associated with whether it is the first marriage or a remarriage. Thus, it is uncertain in this study if older fathers were an independent factor as we did not collect data about the number of marriages.

In this study the maternal mental disorder was particularly strongly associated with child maltreatment. The inspection of the child abuse death in Ministry of Health, Labor and Welfare of Japan also confirmed that the maternal mental disorder was a contributing factor in the child abuse death (5). The maternal mental disorder was also related to other background factors and gestational factors. The presence of a mental disorder was associated with poverty and a deterioration of cognitive function, which may lead to little prenatal care and hypertension due to poor self-health management. In addition, preterm birth or low-birth-weight baby can increase the burden of childcare, which may lead to deteriorate the mother's mental status in the postpartum period.

The gestational factor related to child maltreatment was mothers with a late first visit or little prenatal care. In Japan, prenatal checkups at obstetric facilities are scheduled frequently (at least 14 times). The median number of prenatal checkups in the control group was thirteen, which was significantly higher than ten among the cases. Ten visits may be adequate in some countries; however, skipping scheduled medical checkups for any reason may be presumed to be related to child maltreatment. Moreover, low-birth-weight babies and babies with congenital disease were associated with a significant increase in the odds of subsequent child maltreatment, which was consistent with previous studies (14–16). Risk factors of child maltreatment and perinatal records have been reported to be related. Kelly et al. found an association between perinatal records and subsequent abusive head trauma; the risk factors of abusive head trauma were being young, single, and Maori; having shorter interpregnancy intervals, rupture of membranes for longer than 48 h, and an early gestational age at delivery; and engaging in formula feeding in the first week of life (17). Further, a large UK cohort study, the Avon Longitudinal Study of Parents and Children (ALSPAC), reported the risk factors during pregnancy associated with child maltreatment (7, 18, 19). In their analysis, young parents, low educational achievement, past psychiatric history, a history of childhood abuse, deprivation, and poor social networks were all significantly higher at investigation or registration in the foster home. Moreover, single parents, broken and reordered families, and low-birth-weight children were all at high risk of registration. The factors that were consistent with those in our study were young parents, past psychiatric history, and single parents. Although the status of pregnancy medical examinations was not considered in the ALSPAC study, we found that a late first and few prenatal visits were significantly related to child maltreatment.

Several instruments have been developed for the early identification of families at risk for child maltreatment and their validity has been assessed; for example, the California Family Risk Assessment (CFRA) in the USA or the Instrument for Identification of Parents at Risk for Child Abuse and Neglect (IPARAN) in the Netherlands (20, 21). While there are no such comprehensive national instruments in Japan, some obstetrics hospitals and health organizations evaluate perinatal risk using their own methods.

The strength of this study was that the factors found in this research can be readily adapted throughout Japan as the data were gathered from the MCH, which is standardized in Japan. The MCH was used in some countries other than Japan, and the information described in MCH was easily available and generally versatile.

The following limitations should be considered. First, the subjects in cases were limited to those with their copies of the MCH. The reasons for not having their copies included: not received, lost, or forgotten the MCH. Cases without the MCH may have further problems during pregnancy. Prospective research covering every registered child is required to re-evaluate our results adequately. Second, only the most severely affected children who entered foster homes were included among the cases. A different outcome might be obtained if we investigate children who required the city's care but were not severely affected enough to enter the foster home. Third, despite excluding cases of children in the city's care, undetected child maltreatment cases may have been included in the control group. Fourth, there was selection bias in the control group as the data were obtained from voluntary questionnaires and mothers' self-report. Fifth, we only examined information contained within the MCH at the infant health examination. Thus, this study did not cover some factors that have been reported to be associated with child maltreatment, such as the parent's own childhood abuse, educational qualification, and employment status. Finally, the age ranges differed between the groups; however, the analysis was adjusted for the age of admission to the child protection register or the age at the time of investigation.

After a more extensive investigation across Japan, we aspire to start screening for risk of child maltreatment during pregnancy using these factors and provide education and resources based on risk. Targeted support during pregnancy may prevent the onset of subsequent child maltreatment. We are now starting same research in several region and web research in Japan to use a simpler approach to assess the risk of child maltreatment during pregnancy.

In conclusion, this study showed some of the gestational factors (i.e., mothers with late first visit or little prenatal care), children's factors (i.e., low-birth-weight babies and babies with congenital disease), and background factors (i.e., mother under 25 years old, father over 10 years older than the mother, unmarried mother, maternal mental disorder) were strongly associated with child maltreatment. It would be important to detect mothers and children in need of support throughout the course of pregnancy and the postpartum period.

DATA AVAILABILITY STATEMENT

Data availability access is restricted by the ethics committee. Requests to access the datasets should be directed to HK, haruna@wch.opho.jp.

ETHICS STATEMENT

This research was approved by the Osaka Women's and Children's Hospital's ethical review board (approval number 887, date of approval: March 8th, 2016). Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

HK made the concept of the study design and analyzed and interpreted the patient data regarding prenatal data, and was a major contributor in writing the manuscript. TF, AI, and SD analyzed and interpreted the patient data. YO, NM, and TKa made the concept of the study design. TKi supervised this work. All authors read and approved the final manuscript.

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REFERENCES

1. Shaw M, De Jong M. Child abuse and neglect: a major public health issue and the role of child and adolescent mental health services. *Psychiatrist.* (2012) 36:321–5. doi: 10.1192/pb.bp.111.037135
2. Lueger-Schuster B, Knefel M, Gluck TM, Jagsch R, Kantor V, Weindl D. Child abuse and neglect in institutional settings, cumulative lifetime traumatization, and psychopathological long-term correlates in adult survivors: The Vienna Institutional Abuse Study. *Child Abuse Negl.* (2017) 76:488–501. doi: 10.1016/j.chiabu.2017.12.009
3. Fujiwara T, Okuyama M, Izumi M, Osada Y. The impact of childhood abuse history and domestic violence on the mental health of women in Japan. *Child Abuse Negl.* (2010) 34:267–74. doi: 10.1016/j.chiabu.2009.07.007
4. Stoltenborgh M, Bakermans-Kranenburg MJ, Alink LRA, Ijzendoorn MH. The prevalence of child maltreatment across the globe: review of a series of meta-analyses. *Child Abuse Rev.* (2015) 24:37–50. doi: 10.1002/car.2353
5. Ministry of Health, Labor and Welfare. *Results of Investigation on Child Abuse Deaths (summary of 13th report) and numer of child abuse consultations 2018*. Tokyo: Ministry of Health, Labor and Welfare (2018).
6. Benedict MI, White RB, Cornely DA. Maternal perinatal risk factors and child abuse. *Child Abuse Negl.* (1985) 9:217–24. doi: 10.1016/0145-2134(85)90014-6
7. Sidebotham P, Heron J. Child maltreatment in the "children of the nineties": a cohort study of risk factors. *Child Abuse Negl.* (2006) 30:497–522. doi: 10.1016/j.chiabu.2005.11.005
8. Strathearn L, Mamun AA, Najman JM, O'Callaghan MJ. Does breastfeeding protect against substantiated child abuse and neglect? A 15-year cohort study. *Pediatrics.* (2009) 123:483–93. doi: 10.1542/peds.2007-3546
9. Fujiwara T, Okuyama M, Tsui H, Koenen KC. Perinatal factors associated with infant maltreatment. *Clin Med Insights Pediatr.* (2008) 1:29–36. doi: 10.4137/CMPed.S980
10. Takayanagi K, Iwasaki S, Yoshinaka Y. The role of the maternal and child health handbook system in reducing perinatal mortality in Japan. *Clin Perform Qual Health Care.* (1993) 1:29–33.
11. Suzuki S, Sekizawa A, Tanaka M, Okai T, Kinoshita K. Current status of women requiring perinatal mental health care for protecting their children in Japan. *Asian J Psychiatry.* (2016) 22:93. doi: 10.1016/j.ajp.2016.05.009
12. Minakami H, Maeda T, Fujii T, Hamada H, Iitsuka Y, Itakura A, et al. Guidelines for obstetrical practice in Japan: Japan Society of Obstetrics and Gynecology (JSOG) and Japan Association of Obstetricians and Gynecologists (JAOG) 2014 edition. *J Obstetr Gynaecol Res.* (2014) 40:1469–99. doi: 10.1111/jog.12419
13. Ministry of Health, Labor and Welfare. *Primary Measures for Maternal and Child Health*. Tokyo: Ministry of Health, Labor and Welfare (2009).
14. Windham AM, Rosenberg L, Fuddy L, McFarlane E, Sia C, Duggan AK. Risk of mother-reported child abuse in the first 3 years of life. *Child Abuse Negl.* (2004) 28:645–67. doi: 10.1016/j.chiabu.2004.01.003
15. Wu SS, Ma CX, Carter RL, Ariet M, Feaver EA, Resnick MB, et al. Risk factors for infant maltreatment: a population-based study. *Child Abuse Negl.* (2004) 28:1253–64. doi: 10.1016/j.chiabu.2004.07.005
16. Van Horne BS, Caughy MO, Canfield M, Case AP, Greeley CS, Morgan R, et al. First-time maltreatment in children ages 2-10 with and without specific birth defects: a population-based study. *Child Abuse Negl.* (2018) 84:53–63. doi: 10.1016/j.chiabu.2018.07.003
17. Kelly P, Thompson JMD, Koh J, Ameratunga S, Jelleyman T, Percival TM, et al. Perinatal risk and protective factors for pediatric abusive head trauma: A multicenter case-control study. *J Pediatr.* (2017) 187:240–6.e4. doi: 10.1016/j.jpeds.2017.04.058
18. Sidebotham P, Golding J. Child maltreatment in the "children of the nineties" a longitudinal study of parental risk factors. *Child Abuse Negl.* (2001) 25:1177–200. doi: 10.1016/S0145-2134(01)00261-7
19. Sidebotham P, Heron J, Golding J. Child maltreatment in the "Children of the Nineties:" deprivation, class, and social networks in a UK sample. *Child Abuse Negl.* (2002) 26:1243–59. doi: 10.1016/S0145-2134(02)00415-5
20. Johnson WL. The validity and utility of the California family risk assessment under practice conditions in the field: a prospective study. *Child Abuse Negl.* (2011) 35:18–28. doi: 10.1016/j.chiabu.2010.08.002
21. van der Put CE, Bouwmeester-Landweer MBR, Landsmeer-Beker EA, Wit JM, Dekker FW, Kousemaker NPJ, et al. Screening for potential child maltreatment in parents of a newborn baby: the predictive validity of an instrument for early identification of parents at risk for child abuse and neglect (IPARAN). *Child Abuse Negl.* (2017) 70:160–8. doi: 10.1016/j.chiabu.2017.05.016

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Early Life Domestic Pet Ownership, and the Risk of Pet Sensitization and Atopic Dermatitis in Preschool Children: A Prospective Birth Cohort in Shanghai

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Background: Although domestic pet ownership is on the rise, the impact of early life pet ownership on children's pet sensitization and atopic dermatitis (AD) remains controversial.

Methods: Shanghai Allergy Cohort is an ongoing prospective study followed up to the age of 5 years. Pregnant mothers were recruited and their offspring were followed up every year by a group of pediatricians. Information on furred pet ownership was collected by the questionnaire. AD was diagnosed by dermatologists according to disease history and Williams criteria at 5 years ± 1 months. Skin prick test (SPT) was performed to determine sensitization to specific allergens. Multiple logistic regression models were used to evaluate the associations between pet ownership and AD, dog/cat sensitization.

Results: In the 538 children at preschool age, 112 (20.82%) were diagnosed with AD. Dermatophagoides pteronyssinus and Dermatophagoides farina were the most common allergens, and almost 10% of children were positive to dog and cat. The percentage of positive SPT reactors at 5-year old was 65.28% in the group of children with AD, higher than that in non-AD group (44.57%). Domestic pet ownership at both infant and preschool period was positively associated with an increased risk of sensitization to dog ($OR_{adjusted} = 2.85$ [95% CI: 1.08–7.50 for infant exposure], $OR_{adjusted} = 2.73$ [95% CI: 1.33–5.61] for preschool exposure), and interestingly, pet ownership at infant period negatively associated with higher risk of AD at 5-year old (OR adjusted = 0.33 [95% CI: 0.12–0.88]).

Conclusion: This is the first prospective birth cohort study in Shanghai that found half of preschool children had positive allergen sensitization even in the non-AD children. Although early life exposure to dog may increase the risk of dog sensitization, it significantly decreased the risk of AD. The underlying mechanisms warrant further investigations.

Keywords: pet ownership, dog ownership, atopic dermatitis, allergic sensitization, birth cohort

INTRODUCTION

In the recent decades, the prevalence of atopic dermatitis (AD) in China rapidly increased from 3.07% in 2002 to 8.30% in 2012 and even arrived at 12.94% in 2015 (1–3). Meanwhile, raising a pet especially a dog, becomes more popular among Chinese families in metropolitans like Shanghai. It is reported that domestic animals have become a major source of indoor allergens in urban areas of China (4). However, the prevalence of pet allergen sensitization is limited especially among healthy pre-school children. It's also not clear whether raising pet will increase the risk of allergen sensitization.

Epidemiological evidence of the relationship between pet exposure and risk of AD among children from prospective studies was controversial (5–9). Earlier reviews as well as cohort studies showed a protective effect of pet exposure on the risk of AD in infants or children (9–11). However, other studies found pet-keeping had no or increased effect on allergic disease (12, 13). Ambiguous results have also been shown in previous studies of pet exposure on the risk of pet sensitization, with decreased risk (14, 15), increased risk (16, 17), and no effect (16–18) of such exposure. The inconsistencies or discrepancies among these studies could be due to the differences in the definitions of pet exposure, methods and age of AD diagnosis in children.

In this birth cohort study, with thorough physical examination and lab tests like skin prick test, we aimed to find the impact of furred pet exposure in early life on the risks of AD and pet sensitization.

METHODS

Study Design

The study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures were approved by the institutional review board of Xinhua hospital and the International Peace Maternity and Child Hospital.

Shanghai Allergy Cohort is an ongoing birth cohort study with the pursuit of early life risk factors of allergic risks. A total of 1,053 mother-newborn pairs were enrolled in our study as a baseline from Xinhua Hospital and the International Peace Maternity and Child Hospital affiliated to Shanghai Jiao Tong University between June 2012 and February 2013 in Shanghai, China. The detailed recruitment strategies and characteristics of the cohort have been described previously (19). Briefly, mothers were recruited during pregnancy and children were followed up each year face-to-face or by telephone. At each visit, a standard questionnaire interview was conducted to collect children's information regarding home environmental factors. Of these pairs, 626 children finished face-to-face interviews at 12 months and 743 pairs (70.56%) completed the 5-year follow-up (including 198 by telephone interview). The physical examination was performed in 545 children by a group of physicians including pediatrician, dermatologist, respiratory physician, gastroenterologist, and ophthalmologist. A full skin examination, including recording of all positive signs of skin and rash scoring in different parts of the body, was completed in 538 children who were included in the analysis. The follow up flow chart is shown in **Figure 1**.

Information on maternal educational level, maternal history of allergic disease, prenatal furred pet exposure, maternal passive tobacco smoke exposure during pregnancy, mode of delivery, birthweight, parity, breastfeeding, siblings, and gestational age at birth was collected using self-administered questionnaires. Raising any furred pet, mainly dog, cat and rabbit, indoor for at least 1 month was defined as raising a furred pet. AD was diagnosed according to disease history and the criteria of Williams published in 1994 (20). SPT was performed by dermatologists to test specific allergens, including nine aeroallergens [*Dermatophagoides pteronyssinus(der p)*, *Dermatophagoides farina(der f)*, dog dander, cat dander, *Aspergillus niger*, ragweed pollen, *Platanus hispanica* pollen, birch pollen, and willow pollen] and ten food allergens (egg, milk, mango, prawn, sea crab, beef, mutton, cashew, walnut). Histamine and physiologic saline were used as positive and negative controls, respectively. A positive result was confirmed when diameters of the urticarial weal was at least 3 mm larger than that of negative control. A diagnosis of AD required the presence of the symptom of an itchy rash as well as at least 3 of the following features: (1) history of flexural involvement; (2) onset under the age of 2 years; (3) personal history of asthma or allergic rhinitis; (4) history of a dry skin; and (5) visible flexural dermatitis. The SCORAD index was used to evaluate the severity of AD as previously described by Schmitt (21).

Statistical Analysis

Categorical variables were described using frequencies and percentages. The *chi*-square test was used to evaluate the association between dog sensitization and AD. Non-parametric Mann-Whitney-Wilcoxon test was applied to compare the difference in SCORAD between AD patients with furred pet exposure and those without (21). Associations between pet exposure in pregnancy, infancy, and the preschool period and risk of AD at the age of 5 years were evaluated by multivariate logistic regression models adjusted for sex, maternal atopy, maternal education, mode of delivery, birthweight, parity, gestational age, breastfeeding, siblings and maternal tobacco smoke exposure during pregnancy. The impact of raising pet on allergy sensitizations were also evaluated. All statistical analyses were performed with SPSS (version 20, Chicago, IL, USA).

RESULTS

Demographic Characteristics

A total of 743 (70.56%) children finished the follow-up at 5 years of age; among them, 198 children were followed-up by telephone and 545 by face-to-face interview. We finally included 538 children who finished both the questionnaire and physical examination, 97.03% of these children were born at full-term and 92.57% were first-parity birth (**Table 1**); 71.00% were breastfed to at least 6 months. A total of 11.16% (60/538) owned pets at home during pregnancy, mainly dogs (88.39%). During the first year after birth, 13.96% (55/394) of children had raised pets indoor for at least 1 year, mainly dogs (82.14%, 45/55). During

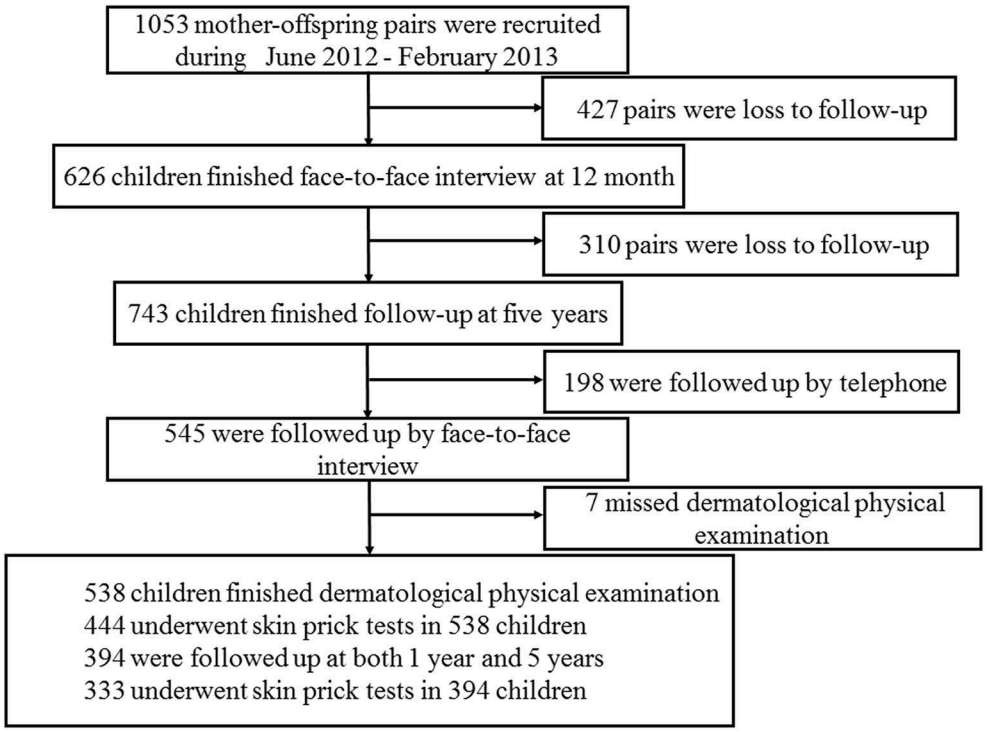

FIGURE 1 | Flow chart of the follow up.

the preschool period, 17.10% of children owned pets at home, and still mainly dogs (55.55%). The number of families with cat exposure was small (0.93%, 5/538) but among them only one child was diagnosed with AD. No differences were found in the characteristics between pet ownership group and non-pet ownership group (**Table 1**).

Prevalence of Positive Allergic Sensitization and Associations Between Early Life Pet Ownership and Allergies Sensitization at Age 5 Years

A total of 444 (82.53%) children underwent skin prick tests. Inhaled allergens were the most common allergen. More than 40% of children were sensitive either to *Der p* (195/444, 43.92%) or to *Der f* (200/444, 45.05%), and about 10% were sensitive to dog dander (42/444, 9.46%) or cat dander (40/444, 9.01%), respectively (**Table 2**). Among AD cases, the proportions of children with positive sensitization to *Der p* as well as *Der f* were about 1.5 times higher than those of the non-AD group. The percentage of children with positive sensitization to dog and cat dander was comparable in AD and non-AD group (**Figure 2**). Percentages of children with a particular number of allergic sensitizations are shown in **Figure 3**. The percentage of SPT positive reactors at 5 years was 65.28% in the group of children with AD, higher than that in the non-AD group (44.57%). The proportion of children who developed sensitization to two or more allergens was 61.11% in AD group, which was also much higher than that in the non-AD group (39.50%). Percentage of SPT negative reactors was lower in AD group than that in non-AD group (34.72 vs. 55.43%).

Pet or dog ownership during the infancy period was associated with increased sensitization to dog ($OR_{adjusted} = 2.85$, [95%CI: 1.08–7.50] for pet ownership and $OR_{adjusted} = 3.44$, [95%CI: 1.31–9.07] for dog ownership) (**Table 3**). The similar positive associations were observed between pet and dog ownership during the preschool period and the incidence of dog allergies ($OR_{adjusted} = 2.73$, [95%CI: 1.33–5.61] for pet ownership and $OR_{adjusted} = 3.64$, [95%CI: 1.57–8.42] for dog ownership). Prenatal pet or dog ownership was also related to increased prevalence of dog allergies. However, the association did not reach statistical significance. Similar situation was found in the association between pet or dog ownership and the prevalence of cat allergies (**Table 3**).

Associations Between Early Life Pet Ownership and AD at 5 Years

Of 538 children, 112 (20.82%) were diagnosed with AD at 5 years of age. As illustrated in **Table 4**, pet ownership during infancy was significantly associated with a lower risk of AD at 5 years ($OR_{adjusted} = 0.33$, 95% CI: 0.12–0.88). The risk of AD was also halved in the group of preschool children exposed to pet during pregnancy and during the preschool period. However, the association did not reach statistical significance possibly due to the limited exposure. These associations persisted after adjustment for covariates as mentioned in methods. When analyzing association between dog ownership and risk of AD, the

TABLE 1 | Demographic characteristics of mother-child pairs in Shanghai Allergy Cohort by pet owner.

Characteristics	N (%)	Non-Pet owner	Pet owner	*p*
N	538	447	92	
Boys	272 (50.56)	224 (50.11)	42 (45.65)	0.41
Maternal educational level				0.15
Lower than bachelor	75 (13.94)	61 (13.68)	13 (14.13)	
Bachelor	411 (76.39)	336 (75.33)	75 (81.52)	
Higher than bachelor	53 (9.85)	49 (10.99)	4 (4.35)	
Mode of delivery				0.46
Vaginal delivery	139 (25.84)	118 (26.58)	21 (22.82)	
Cesarean section	397 (74.1)	326 (73.42)	71 (77.17)	
Birth Weight				0.53
<2,500 g	14 (2.61)	12 (2.70)	2 (2.17)	
2,500–4,000 g	472 (87.90)	388 (87.19)	84 (91.3)	
>=4,000 g	51 (9.50)	45 (10.11)	6 (6.52)	
Primiparity	498 (92.57)	413 (92.8)	85(92.4)	0.89
Gestational age				0.87
≥ 37 weeks	522 (97.03)	432 (96.86)	90 (97.83)	
< 37 weeks	16 (2.97)	14 (3.14)	2 (2.17)	
Passive smoking during pregnancy	164 (30.48)	130 (29.08)	34 (36.96)	0.34
Maternal atopy	87 (16.17)	73 (16.33)	15 (16.30)	0.97
Breastfeeding before 6 months	382 (71.00)	319 (71.36)	63 (68.48)	1.00

TABLE 2 | Prevalence of positive allergic sensitization in all children, atopic dermatitis group, and non-atopic dermatitis group.

Allergens	Total (%)	AD-group (%)	Non AD-group (%)
Dermatophagoides pteronyssinus	43.92	60.61	39.13
Dermatophagoides farina	45.05	61.62	40.29
Dog	9.46	11.11	8.99
Cat	9.01	8.08	9.28
Aspergillus niger	2.93	6.06	2.03
Ragweed pollen	1.35	1.01	1.45
Platanus hispanica pollen	1.35	0.00	1.74
Birch pollen	2.03	3.03	1.74
Willow pollen	3.38	7.07	2.32
Egg	3.38	8.08	2.03
Milk	2.25	4.04	1.74
Mango	0.90	3.03	0.29
Prawn	2.25	8.08	0.58
Sea crab	1.80	6.06	0.58
Beef	1.13	1.01	1.16
Mutton	0.90	1.01	0.87
Cashew	0.90	3.03	0.29
Walnut	2.48	4.04	2.03

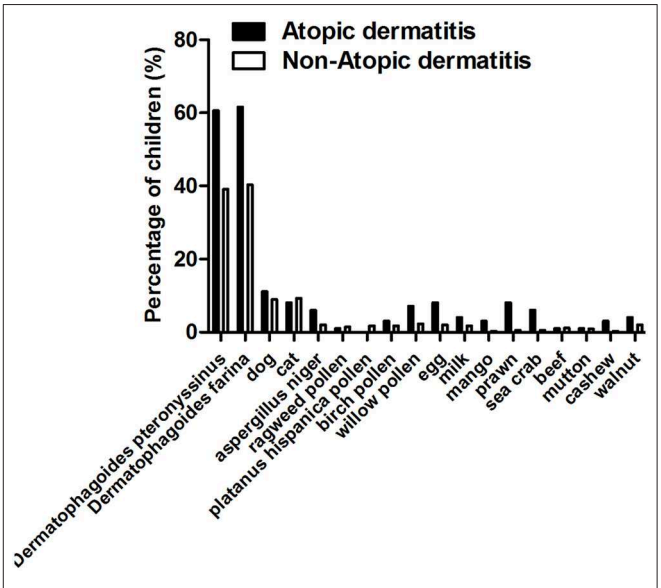

FIGURE 2 | Frequency of positive allergic sensitization in Shanghai Allergy Cohort stratified by AD. The particular type of allergy sensitization in each group was calculated. Percentages of children with positive allergic sensitization in either group were analyzed. The x-axis shows the particular type of allergy sensitization. The y-axis shows the percentages of children with positive allergic sensitization in either group.

trend was similar to that between pet ownership and risk of AD. We also evaluated whether pet ownership could affect the severity of AD. The average SCORAD index of patients with pet exposure during infancy was 18.49 (range: 0.02–35.10) and without pet exposure was 17.15 (range: 6.27–40.40). No difference was found in the SCORAD index between children with pet exposure during infancy and those without ($p = 0.39$, $p > 0.05$), neither between children with and without dog exposure (19.40, 9.90–35.10 vs. 16.99, 0.02–40.40) ($p = 0.15$, $p > 0.05$). Similar non-significant results were also found between AD children with pet exposure and those without exposure at pregnancy and in preschool period.

We further analyzed the association between dog sensitization and AD according to dog ownership (**Table 5**). Among children with dog ownership, the percentage of AD was much lower in the group with dog sensitization than those without (**Table 5**). Dog allergies decreased the risk of AD in children who raised dog at home (OR = 0.82, [95%CI: 0.71–0.94] for pregnancy exposure; the OR was 0.89, [95%CI: 0.79–1.00] for infancy exposure and 0.78 [95%CI: 0.66–0.93] for preschool exposure), but the dog sensitization had no association with the risk of AD in children without dog ownership (**Table 5**). However, no significant difference was found between cat sensitization and AD according to cat ownership (data not shown).

DISCUSSION

This is the first prospective birth cohort study to investigate the relationship between pet ownership and allergic sensitization and AD in Chinese population. We found that more than 40% of children had positive allergic sensitization in Shanghai, and almost one fifth of them suffered from AD. Children with infancy and preschool pet and dog ownership tended to have the dog sensitization. However, pet ownership during infancy

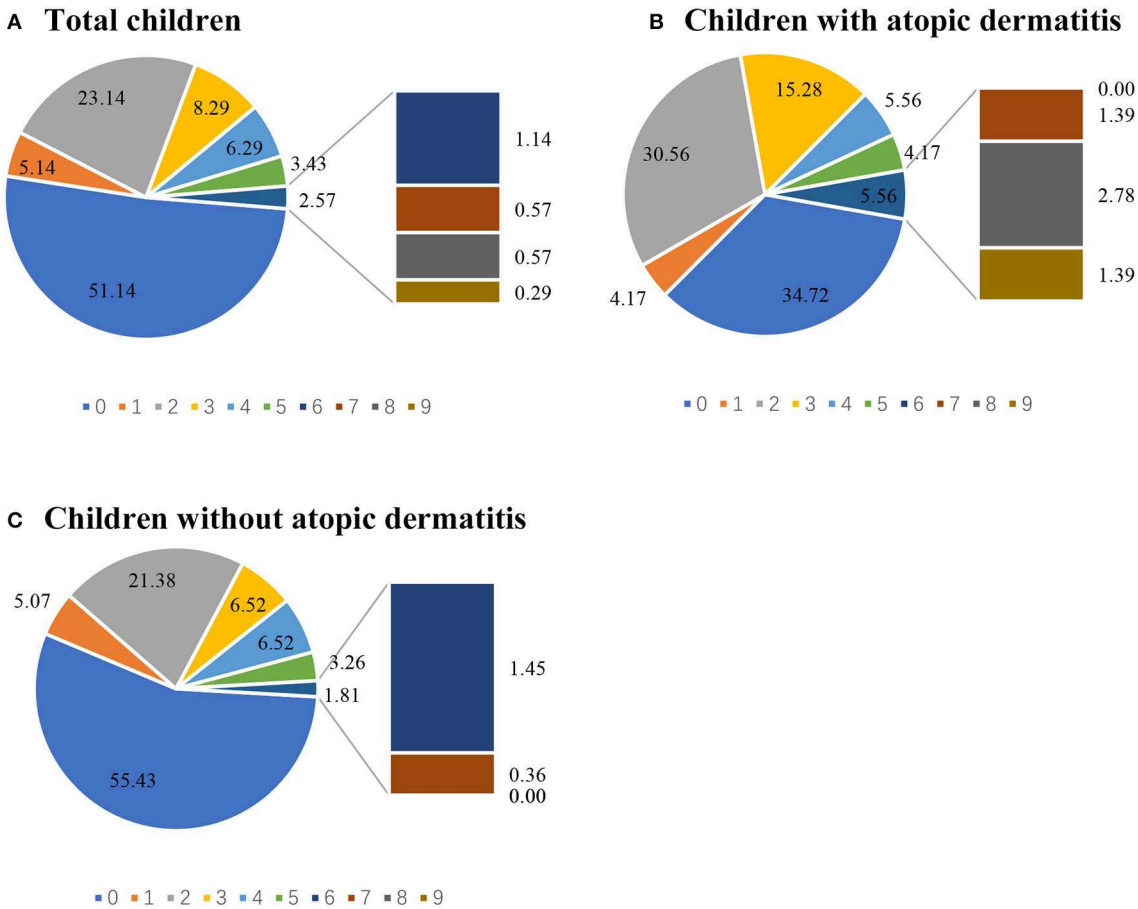

FIGURE 3 | The positive number of allergic sensitization in each child was calculated. Percentages of children with particular number of allergic sensitization were analyzed in all children with skin prick test **(A)**, children with AD and underwent skin prick test **(B)**, and children without AD and underwent skin prick test **(C)** which was calculated as percentage(%)=(number of children with particular number of allergic sensitization)/(the number of children in each group who underwent the skin prick test) × 100%.

was associated with a lower risk of developing AD at age 5 years.

To date, no birth cohort study in Shanghai had reported epidemiological data of allergic sensitization. Dermatophagoides pteronyssinus or Dermatophagoides farina were the most common allergens in our study, similar to previously reported data in Asian countries. (22) Dog and cat dander sensitization ranked the second. In a previous cross-sectional study in Shanghai, mite allergy was also the most common allergen (17.6%), followed by pollen (12.8%), and food allergy (12.3%) (23). Prevalence of overall European rates of sensitization to cat allergens were 26.3%, ranging from 16.8 to 49.3% and to dog allergens 27.2%, ranging from 16.1 to 56.0%, much higher than that to Dermatophagoides pteronyssinus and Dermatophagoides farina (1–7%). (24) Overall, the percentage of SPT positive reactors in the group of children with AD was higher than that in non-AD group. Cumulative evidence revealed that dog exposure in early childhood prevents the development of allergies to dogs and cats (5, 12, 13). However, a positive association between postnatal pet exposure and the prevalence of dog sensitization was found in our study, which was consistent with previous data (16, 17, 25, 26).

It is reported that pet-or dog-ownership during pregnancy significantly lowered the risk of AD in the year of 1 or 2 in two previous prospective birth cohort studies (10, 27). Roduit et al. found that children with prenatal pet and cat contact had a lower risk of AD in the first 2 years of life than those without (10). Another study regarding prenatal maternal exposure to dogs at home was "protective" against allergic diseases (27). Similarly, the risk of AD was also lower in the group of pet ownership during pregnancy in this study although it did not reach statistical significance. It is possibly due to the small number of children with prenatal pet and dog exposure.

Inconsistent results were found in correlation between postnatal pet exposure and development of allergic diseases in previous studies (5–7, 12, 13, 28–31). The probable causes for these discrepancies could be the differences in the timing of exposures, the way pets are kept and the diagnosis criteria of AD (11). Pet exposure was not well-measured as pet contact was considered as pet exposure in some studies, which was

TABLE 3 | Associations between early life pet ownership and the risk of sensitization to dog and cat at 5 years of age.

	Sensitization outcome at age of 5 years					
	Dog sensitization			Cat sensitization		
	N(%)	Crude ORs (95%CIs)	Adjusted ORs (95%CIs)	N(%)	Crude ORs (95%CIs)	Adjusted ORs (95%CIs)
PET OWNERSHIP						
During pregnancy	6 (11.54)	1.31 (0.52–3.29)	1.48 (0.56–3.90)	6 (11.54)	1.36 (0.54–3.39)	1.36 (0.52–3.55)
Infant period	8 (16.00)	**2.50 (1.03–6.03)**	**2.85 (1.08–7.50)**	8 (16.00)	**2.50 (1.03–6.03)**	2.15 (0.82–5.46)
Preschool period	14 (18.18)	**2.67 (1.33–5.35)**	**2.73 (1.33–5.61)**	9 (11.69)	1.42 (0.65–3.12)	1.43 (0.64–3.17)
DOG OWNERSHIP						
During pregnancy	6 (13.04)	1.50 (0.59–3.77)	1.73 (0.65–4.64)	6 (13.04)	1.59 (0.63–4.03)	1.58 (0.60–4.17)
Infant period	8 (18.18)	**2.98 (1.22–7.26)**	**3.44 (1.31–9.07)**	8 (18.18)	**2.98 (1.22–7.26)**	**2.72 (1.07–6.90)**
Preschool period	10 (22.22)	**3.25 (1.48–7.16)**	**3.64 (1.57–8.42)**	6 (13.33)	1.64 (0.65–4.15)	1.62 (0.63–4.17)

Adjusted OR: calculated by the logistic models adjusted for sex, maternal atopy, maternal education, mode of delivery, birthweight, parity, gestational age, and smoking during pregnancy. Bold values indicate with statistical significance.

TABLE 4 | Associations between early life pet ownership and the risk of atopic dermatitis at 5 years of age.

	N(%)	Crude ORs	95% CI	Adjusted ORs	95% CI
Pet ownership					
During pregnancy	8 (13.33)	0.47	0.21–1.05	0.51	0.22–1.18
Infant period	6 (10.91)	**0.34**	**0.13–0.88**	**0.33**	**0.12–0.88**
Preschool period	14 (15.22)	0.64	0.35–1.17	0.62	0.33–1.16
Dog Ownership					
During pregnancy	7 (13.21)	0.55	0.24–1.26	0.60	0.25–1.40
Infant period	5 (10.64)	**0.32**	**0.11–0.92**	**0.31**	**0.11–0.91**
Preschool period	9 (17.65)	0.8	0.38–1.69	0.72	0.33–1.57

Pet ownership was defined as the presence of any furred pet living in the house at the time of visit.
Adjusted OR: logistic models adjusted for sex, maternal atopy, maternal education, mode of delivery, birthweight, parity, gestational age, and passive smoking during pregnancy. Bold values indicate with statistical significance.

TABLE 5 | Association between dog sensitization and risk of AD at age of 5 years according to the dog ownership status (YES/NO) in early life.

	Dog sensitization at 5 years of age		OR (95% CI)
	Yes (Cases/N)	No (Cases/N)	
Dog ownership			
During pregnancy	0/6	6/41	**0.82 (0.71–0.94)**
Infant period	0/8	5/36	**0.89 (0.79–1.00)**
Preschool period	2/10	6/35	**0.78 (0.66–0.93)**
No dog ownership			
During pregnancy	11/36	82/361	0.66 (0.32–1.41)
Infant period	8/20	59/269	0.43 (0.17–1.11)
Preschool period	9/32	82/367	0.74 (0.33–1.65)

Bold values indicate with statistical significance.

very difficult to quantify. The exposure window span was too wide from pregnancy to infancy and even the toddler period. In addition, the diagnosis of AD in previous literature was mostly measured by questionnaire (32). It's hard to make diagnosis correctly without disease history and physical examination. In this birth cohort study, with thorough skin physical examination and lab tests like skin prick test, we diagnosed AD and found a significant association between pet ownership during the infancy period and the prevalence of AD.

Early life represents a critical window for immune development. During this period, a variety of environmental factors, such as pet exposure, encountered by infants determine health or allergic diseases (33). Several protective mechanisms of early life pet exposure had been summarized as follows. Exposure to endotoxin has been speculated as the cause behind the protective effect, which may shift the developing immune system to predominantly Th1-type responses that protect children from developing allergy (34–37). The study by Gern et al. observed the association between dog ownership and higher serum IL-10 and IL-13 cytokine secretion in children at age 1 (5). Cytokine balance during early life, which could be influenced by external factors, is critical in the development of allergic response (30). In addition, recent studies attempting to correlate genetic variations in Toll-like receptors (TLRs) and CD14 genes with atopic disease supported the existence of a gene-environment interaction, although the results were conflicting (5, 10, 33). However, research by Swedish BAMSE presented that the observed protective effect of dog exposure may be partly due to selective dog avoidance by families with parental atopic dermatitis (38).

In our study, pet ownership during infancy was associated with a lower risk of children AD at age 5 years, but with a higher risk of dog sensitization. Constant exposure to high levels of animal antigen over time might induce tolerance to relevant allergen in sensitized children via desensitization of mast cells and basophils, generation of allergen-specific Treg and Breg cells, inhibition of Th2 cells and other allergen-specific effector T cells. It also could be via the regulation of antibody isotypes with increase in specific IgG4 and decrease or no change in specific IgE (39). Dog sensitization may persist with constant

exposure to animals, but children will not develop AD. The relationship between pet exposure, dog sensitization and the prevalence of AD in our study may be explained by the above-mentioned mechanisms.

There are some limitations in this study. As it usually occurs in other longitudinal cohort studies, some participants were lost during the follow-up, resulting in a relatively small sample size in this cohort. The follow up rate was only 70.56% in our study. The number of families with cat/dog exposure was also small in this study, which may cause deviations to the results. In addition, although we adjusted for a wide range of potential confounders including maternal atopy, possible existence of previous family history of allergic disease can cause bias. Another limitation is that this study failed to explain the observed effect of pet ownership on sensitization and some subjects did not undergo the SPT test. Finally, there may be some residual confounding factors that the data do not allow us to adjust, although several potential confounders had been analyzed. All data were collected prospectively, and hence, recall bias of the information did not exist.

In conclusion, the present cohort study found that pet ownership in early childhood might potentially protect children from the development of AD up to the age of 5 years. Postnatal exposure to a pet at home increased the risk of sensitization to dogs. Further well-designed, large scaled, prospective investigations on the protective effect of pet ownership are needed.

DATA AVAILABILITY STATEMENT

The datasets generated and/or analyzed during the current study are not publicly available due to management rules by the study funder but are available from the corresponding author on reasonable request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Bioethical Committee of the institutional review board of Xinhua hospital and the International Peace Maternity and Child Hospital, shanghai, China. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

CL: conceptualization, methodology, original draft preparation, and funding acquisition. QC: conceptualization, methodology, software, formal analysis, and data curation. XZ, HL, QL, and PF: investigation, validation, writing—review, and editing. LH and ZY: funding acquisition, supervision, and project administration.

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REFERENCES

1. Guo Y, Li P, Tang J, Han X, Zou X, Xu G, et al. Prevalence of atopic dermatitis in Chinese children aged 1-7 ys. *Sci Rep*. (2016) 6:29751. doi: 10.1038/srep29751
2. Yang YC, Cheng YW, Lai CS, Chen W. Prevalence of childhood acne, ephelides, warts, atopic dermatitis, psoriasis, alopecia areata and keloid in Kaohsiung County, Taiwan: a community-based clinical survey. *J Eur Acad Dermatol Venereol*. (2007) 21:643–9. doi: 10.1111/j.1468-3083.2006.02036.x
3. Xu F, Yan S, Li F, Cai M, Chai W, Wu M, et al. Prevalence of childhood atopic dermatitis: an urban and rural community-based study in Shanghai, China. *PLoS ONE*. (2012) 7:e36174. doi: 10.1371/journal.pone.0036174
4. Weidinger S, Novak N. Atopic dermatitis. *Lancet*. (2016) 387:1109–22. doi: 10.1016/S0140-6736(15)00149-X
5. Gern JE, Reardon CL, Hoffjan S, Nicolae D, Li Z, Roberg KA, et al. Effects of dog ownership and genotype on immune development and atopy in infancy. *J Allergy Clin Immunol*. (2004) 113:307–14. doi: 10.1016/j.jaci.2003.11.017
6. Epstein TG, Bernstein DI, Levin L, Khurana Hershey GK, Ryan PH, Reponen T, et al. Opposing effects of cat and dog ownership and allergic sensitization on eczema in an atopic birth cohort. *J Pediatr*. (2011) 158:265–71. doi: 10.1016/j.jpeds.2010.07.026
7. Thorsteinsdottir S, Thyssen JP, Stokholm J, Vissing NH, Waage J, Bisgaard H. Domestic dog exposure at birth reduces the incidence of atopic dermatitis. *Allergy*. (2016) 71:1736–44. doi: 10.1111/all.12980
8. Bornehag CG, Sundell J, Hagerhed L, Janson S, DBH Study Group. Pet-keeping in early childhood and airway, nose and skin symptoms later in life. *Allergy*. (2003) 58:939–44. doi: 10.1034/j.1398-9995.2003.00050.x
9. Pelucchi C, Galeone C, Bach JF, La Vecchia C, Chatenoud L. Pet exposure and risk of atopic dermatitis at the pediatric age: a meta-analysis of birth cohort studies. *J Allergy Clin Immunol*. (2013) 132:616–22. doi: 10.1016/j.jaci.2013.04.009
10. Roduit C, Wohlgensinger J, Frei R, Bitter S, Bieli C, Loeliger S, et al. Prenatal animal contact and gene expression of innate immunity receptors at birth are associated with atopic dermatitis. *J Allergy Clin Immunol*. (2011) 127:179–85. doi: 10.1016/j.jaci.2010.10.010
11. Lodge CJ, Allen KJ, Lowe AJ, Hill DJ, Hosking CS, Abramson MJ, et al. Perinatal cat and dog exposure and the risk of asthma and allergy in the Urban environment: a systematic review of longitudinal studies. *Clin Dev Immunol*. (2012) 2012:176484. doi: 10.1155/2012/176484
12. Almqvist C, Garden F, Kemp AS, Li Q, Crisafulli D, Tovey ER, et al. Effects of early cat or dog ownership on sensitisation and asthma in a high-risk cohort without disease-related modification of exposure. *Paediatr Perinat Epidemiol*. (2010) 24:171–8. doi: 10.1111/j.1365-3016.2010.01095.x
13. Chen CM, Morgenstern V, Bischof W, Herbarth O, Borte M, Behrendt H, et al. Dog ownership and contact during childhood and later allergy development. *Eur Respir J*. (2008) 31:963–73. doi: 10.1183/09031936.00092807
14. Ownby DR, Johnson CC, Peterson EL. Exposure to dogs and cats in the first year of life and risk of allergic sensitization at 6 to 7 years of age. *JAMA*. (2002) 288:963–72. doi: 10.1001/jama.288.8.963
15. Perzanowski MS1, Rönmark E, Platts-Mills TA, Lundbäck B. Effect of cat and dog ownership on sensitization and development of asthma among preteenage children. *Am J Respir Crit Care Med*. (2002) 166:696–702. doi: 10.1164/rccm.2201035

16. Carlsten C, Dimich-Ward H, Becker AB, Ferguson A, Chan HW, DyBuncio A, et al. Indoor allergen exposure, sensitization, and development of asthma in a high-risk birth cohort. *Pediatr Allergy Immunol*. (2010) 21:e740–6. doi: 10.1111/j.1399-3038.2010.01021.x
17. Williams AH, Smith JT, Hudgens EE, Rhoney S, Ozkaynak H, Hamilton RG, et al. Allergens in household dust and serological indicators of atopy and sensitization in Detroit children with history-based evidence of asthma. *J Asthma*. (2011) 48:674–84. doi: 10.3109/02770903.2011.599909
18. Schoos AM, Chawes BL, Jelding-Dannemand E, Elfman LB, Bisgaard H. Early indoor aeroallergen exposure is not associated with development of sensitization or allergic rhinitis in high-risk children. *Allergy*. (2016) 71:684–91. doi: 10.1111/all.12853
19. Wang X, Gao X, Yang Q, Wang X, Li S, Jiang F, et al. Sleep disorders and allergic diseases in Chinese toddlers. *Sleep Med*. (2017) 37:174–9. doi: 10.1016/j.sleep.2017.02.011
20. Williams HC, Burney PG, Pembroke AC, Hay RJ. The UK working party's diagnostic criteria for atopic dermatitis. III. Independent hospital validation. *Br J Dermatol*. (1994) 131:406–16. doi: 10.1111/j.1365-2133.1994.tb08532.x
21. Schmitt J, Langan S, Williams HC, European Dermato-epidemiology network. What are the best outcome measurements for atopic eczema? A systematic review. *J Allergy Clin Immunol*. (2007) 120:1389–98. doi: 10.1016/j.jaci.2007.08.011
22. Tham EH, Lee AJ, Bever HV. Aeroallergen sensitization and allergic disease phenotypes in Asia. *Asian Pac J Allergy Immunol*. (2016) 34:181–9. doi: 10.12932/ap0770
23. Feng Y, Wang X, Wang F, Liu R, Chen L, Wu S, et al. The prevalence of ocular allergy and comorbidities in Chinese school children in Shanghai. *Biomed Res Int*. (2017) 2017:7190987. doi: 10.1155/2017/7190987
24. Zahradnik E, Raulf M. Animal allergens and their presence in the environment. *Front Immunol*. (2014) 5:76. doi: 10.3389/fimmu.2014.00076
25. Al-Mousawi MS, Lovel H, Behbehani N, Arifhodzic N, Woodcock A, Custovic A. Asthma and sensitization in a community with low indoor allergen levels and low pet-keeping frequency. *J Allergy Clin Immunol*. (2004) 114:1389–94. doi: 10.1016/j.jaci.2004.09.005
26. Pyrhönen K, Näyhä S, Läärä E. Dog and cat exposure and respective pet allergy in early childhood. *Pediatr Allergy Immunol*. (2015) 26:247–55. doi: 10.1111/pai.12369
27. Batool T, Reece PL, Schulze KM, Morrison KM, Atkinson SA, Anand SS, et al. Prenatal and early-life predictors of atopy and allergic disease in Canadian children: results of the family atherosclerosis monitoring in earLY life (FAMILY) study. *J Dev Orig Health Dis*. (2016) 7:665–71. doi: 10.1017/S2040174416000386
28. Bufford JD, Reardon CL, Li Z, Roberg KA, DaSilva D, Eggleston PA, et al. Effects of dog ownership in early childhood on immune development and atopic diseases. *Clin Exp Allergy*. (2008) 38:1635–43. doi: 10.1111/j.1365-2222.2008.03018.x
29. Ludvigsson JF, Mostrom M, Ludvigsson J, Duchen K. Exclusive breastfeeding and risk of atopic dermatitis in some 8300 infants. *Pediatr Allergy Immunol*. (2005) 16:201–8. doi: 10.1111/j.1399-3038.2005.00257.x
30. Biagini Myers JM, Wang N, LeMasters GK, Bernstein DI, Epstein TG, Lindsey MA, et al. Genetic and environmental risk factors for childhood eczema development and allergic sensitization in the CCAAPS cohort. *J Invest Dermatol*. (2010) 130:430–7. doi: 10.1038/jid.2009.300
31. Clausen M, Kristjansson S, Haraldsson A, Björkstén B. High prevalence of allergic diseases and sensitization in a low allergen country. *Acta Paediatr*. (2008) 97:1216–20. doi: 10.1111/j.1651-2227.2008.00887.x
32. Prescott SL, Smith P, Tang M, Palmer DJ, Sinn J, Huntley SJ, et al. The importance of early complementary feeding in the development of oral tolerance: concerns and controversies. *Pediatr Allergy Immunol*. (2008) 19:375–80. doi: 10.1111/j.1399-3038.2008.00718.x
33. Lloyd CM, Saglani S. Development of allergic immunity in early life. *Immunol Rev*. (2017) 278:101–15. doi: 10.1111/imr.12562
34. Waser M, von Mutius E, Riedler J, Nowak D, Maisch S, Carr D, et al. Exposure to pets, and the association with hay fever, asthma, and atopic sensitization in rural children. *Allergy*. (2005) 60:177–84. doi: 10.1111/j.1398-9995.2004.00645.x
35. Braun-Fahrländer C, Riedler J, Herz U, Eder W, Waser M, Grize L, et al. Environmental exposure to endotoxin and its relation to asthma in school-age children. *N Engl J Med*. (2002) 347:869–77. doi: 10.1056/NEJMoa020057
36. Schram D, Doekes G, Boeve M, Douwes J, Riedler J, Ublagger E, et al. Bacterial and fungal components in house dust of farm children, Rudolf Steiner school children and reference children–the PARSIFAL study. *Allergy*. (2005) 60:611–8. doi: 10.1111/j.1398-9995.2005.00748.x
37. von Mutius E. Environmental factors influencing the development and progression of pediatric asthma. *J Allergy Clin Immunol*. (2002) 109(6 Suppl.):S525–32. doi: 10.1067/mai.2002.124565
38. Almqvist C, Egmar AC, van Hage-Hamsten M, Berglind N, Pershagen G, Nordvall SL, et al. Heredity, pet ownership, and confounding control in a population-based birth cohort. *J Allergy Clin Immunol*. (2003) 111:800–6. doi: 10.1067/mai.2003.1334
39. van de Veen W, Wirz OF, Globinska A, Akdis M. Novel mechanisms in immune tolerance to allergens during natural allergen exposure and allergen-specific immunotherapy. *Curr Opin Immunol*. (2017) 48:74–81. doi: 10.1016/j.coi.2017.08.012

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Reducing Anemia Among School-Aged Children in China by Eliminating the Geographic Disparity and Ameliorating Stunting: Evidence From a National Survey

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Background: The aim of this study was to assess the geographic disparity in anemia and whether stunting was associated with anemia in different geographic groups among school-aged children in China.

Methods: 71,129 Han children aged 7, 9, 12, and 14 years old were extracted from the 2014 cycle of Chinese National Surveys on Children Constitution and Health. Anemia, anemia severity, and stunting were defined according to WHO definitions. Binary logistic regression models were used to estimate the association between anemia and stunting in different geographic groups.

Results: The prevalence of anemia was significantly higher in girls (10.8%) than boys (7.0%). The highest anemia prevalence was in Group VII (lower class/rural, 12.0%). A moderate/severe prevalence of anemia was concentrated in Group VII and Group VIII (western/lower class/rural) for both sexes. The prevalence of anemia was higher in stunting boys than non-stunting boys in Group IV (lower class/city, $\chi^2 = 12.78$, $P = 0.002$) and Group VII ($\chi^2 = 6.21$, $P = 0.018$), while for girls, it was higher in stunting girls than their non-stunting peers only in Group II (upper class/large city, $\chi^2 = 4.57$, $P = 0.046$). Logistic regression showed that the stunting children have 30% higher risk of anemia than non-stunting children after adjustment for age, sex and school (OR = 1.30, 95% CI: 1.05–1.60).

Conclusion: A significant geographic disparity and an association between anemia and stunting among specific groups of school-aged children in China was demonstrated. Consequently, eliminating the geographic disparity and ameliorating stunting might contribute to the improvement of Chinese children's anemia. Specific guidelines and interventions are needed, especially for adolescent girls and the groups with serious anemia burden.

Keywords: anemia, geographic disparity, stunting, children, China

INTRODUCTION

Anemia, or low concentrations of hemoglobin (Hb), adversely affects cognitive and motor development and study capacity, and increases susceptibility to infection, which also exerts a substantial economic burden on the government (1, 2). Globally, 1.62 billion people suffer with anemia and the prevalence among children was 25.4%, according to a World Health Organization (WHO) (3) report. Asia is the hardest hit, especially in South Asia (4, 5), where an improvement in children's hemoglobin status may lead to a modest global increase in mean hemoglobin and a reduction in anemia prevalence. Globally, anemia is affecting people in both developed and developing countries with different health risks, and almost all age groups and both sexes are susceptible. Even for developed countries, such as Sweden, France, Australia, Denmark, Belgium, and Ireland, anemia prevalence has changed little during past two decades (6). Although iron deficiency anemia is the true indicator of poor nutritional status, considering that 90–95% of anemia cases in China are due to iron deficiency (7), anemia remains useful as an indicator of undernutrition and is particularly relevant for adolescents in the context of rapid growth and menstruation. Compared with children under five and pregnant women, adolescents have not been paid much attention. In 2016, 333 million adolescents with anemia lived in multi-burden settings, of which 194 million lived in India and China according to GBD (Global Burden of Disease study) data (6). Although the prevalence of anemia in China has declined in the past two decades at the national level (8), it was as high as 14.8% in some rural regions like Shanxi (9, 10). Nationwide, more than 19.2 million Chinese children were anemic and 389,198 had severe anemia in 2010, based on a total number of children aged 7–18 years (194,599,052). Nevertheless, updated information of geographic disparity on anemia among Chinese children is unclear. This information is urgently needed as it may provide a solid foundation to alleviate the geographic disparity of anemia among Chinese children.

In China, most anemia is due to prolonged iron deficiency, which impairs hemoglobin production and limits the amount of oxygen that red blood cells carry throughout the body and to the brain (11). Stunting is also a big issue in China and warrants monitoring because it is an undervalued indicator which reflects the cumulative effect of undernutrition with socioeconomic and other factors. These factors may contribute to anemia. Studies in Haiti and Angola confirmed that stunting increased the risks of developing anemia (12, 13). The question is still uncertain as to whether stunting is associated with anemia in China, especially in school-aged children who have been largely under-investigated. Moreover, if the association exists, does it vary or remain similar in different settings and populations?

Hemoglobin data from children aged 7 to 14 of both sexes across China was collected in the 2014 Chinese National Surveys on Students' Constitution and Health (CNSSCH). It provided a valuable opportunity to update the available information on anemia among school-aged children who have been largely neglected in research in China, compared with numerous publications on children under five and pregnant women (14). The objectives of the present study were to: (1) delineate the geographic disparity of anemia; (2) identify the most susceptible population with the heaviest burden of anemia; and (3) examine the association between anemia and stunting, and the relationships across different geographic groups.

MATERIALS AND METHODS

Data was extracted from the 2014 cycle of CNSSCH, which was a large-scale cross-sectional survey of school-aged children conducted by six relevant ministries including the Ministries of Education, Health, Science and Technology, the State Ethnic Affairs Commission, and the State Sports General Administration, China. It spanned 31 provinces, excluding Hong Kong, Macau, and Taiwan. The sampling procedure, as previously described in detail (14), was the same in all CNSSCH survey sites. In brief, this survey was to investigate children's health status in China and used a multistage stratified cluster sampling design (14). In the first stage of sampling, in order to achieve better representation within the 30 provinces, populations were stratified by three socioeconomic indicator groups or three sets of prefecture-level cities (i.e., upper, moderate, low) at the regional level, defined by regional gross domestic product, total yearly income per capita, average food consumption per capita, natural growth rate of the population, and the regional social welfare index. In each group of three sub-provincial levels, one city was selected randomly and remained constant from the first survey in 1985. Within these sub-province regions, populations were also stratified by urban and rural area of residence. Within these stratified areas, a random selection of schools, including primary school, middle school, and high school, was conducted according to the established procedures. In the second stage, sampling took place in classes (primary sampling units or clusters) selected randomly from each grade in these schools, and all students in the selected class were included and listed in the investigation after meeting the inclusion criteria and after obtaining verbally informed consent from both students and their parents. Finally, within the primary sampling units, namely every age from 7 to 18 years for boys and girls, at least 50 Han ethnicity students, the minimum sample size, were included in the survey and sampling yielded equal numbers of the three socioeconomic indicator groups. Thus, the sample weight remained consistent in each age, sex, region (urban/rural), city (three socioeconomic indicators' groups at sub-province level), and province for students aged 7–18 years in each survey year. The participants in this study were Han children aged 7, 9, 12, and 14 years old from 26 provinces and four municipalities, except for Tibet (where the Han ethnicity is a minority). The children were recruited in this study if their parents and themselves had lived in their local regions more than a year.

Participants underwent a medical examination prior to the national survey and were excluded if they had one or more of the following conditions: (1) serious organ disease (e.g., heart, lung, liver, kidney); (2) abnormal physical development (e.g., pygmyism, gigantism); (3) physical impairment or deformity (e.g., severe scoliosis, pectus carinatum, limp, genu valgum,

and gunu varum); or (4) acute disease symptoms (e.g., diarrhea, fever) during the past month and not yet recovered. Consequently, 71,129 children with complete data records on age, sex, urban/rural groups, height, weight and hemoglobin concentration were included in the analysis. Moreover, the ratio of boys/girls or urban/rural groups was approximately equal to 1:1 of each sex- and age-specific subgroup. The project was approved by the Medical Research Ethics Committee of Peking University Health Science Center (IRB00001052-18002). With data collected from schools across China, the school principals were able to determine the process for gaining informed consent from children's parents or guardians. All participants' information was anonymized and de-identified prior to analysis to protect their privacy.

Measures

Participants were required to wear light clothing and stand straight, barefoot, and at ease when height was measured. The heel, humerus, and shoulders were contacted to form a "three-point, one-line" standing position. Measurements were conducted by a team of trained field professionals who were required to pass a training course in anthropometric measurements. Height was measured to the nearest 0.1 cm with a portable stadiometer. The stadiometers were calibrated before use. The measurements were carried out at the same time of the day during the survey (better to be specific, e.g., morning, afternoon, during school recess, etc.). Height-for-age Z-score (HAZ) was calculated by using WHO 2007 references with the fixed population. Stunting was defined using the growth references of HAZ: stunting: <-2SD (15, 16).

Hemoglobin concentration was measured by laboratory technicians for the participants in the selected school. Hemoglobin concentration was measured by HemoCue201+ (Origin: Sweden, Model: HemoCue201$^+$, Manufacturer: HemoCue AB) (17). Data collection was supervised as follows by well-trained on-site investigators: (a) the capillary blood sample was collected from the fingertip after discarding the first drop, and a small amount of blood was pressed out onto the fingertips. The blood was taken continuously with a micro cuvette, (b) the micro cuvette was inserted into the cuvette tank and reacted for 15s to 60s, (c) the score was determined and recorded on site (18). Age specific cut-off values of hemoglobin concentration were used to define anemia. Hemoglobin concentration was defined by using WHO criteria (19, 20) and categorized as: (1) for children aged 5 to 11 year: $\geq$115 g/L normal, $<$115 g/L anemia, 110–114 g/L mild anemia, $\leq$109 moderate/severe prevalence anemia; (2) for children aged 12–14 year: $\geq$120 g/L normal, $<$120 g/L anemia, 110–119 g/L mild anemia, $\leq$109 g/L moderate/severe prevalence anemia.

In order to compare the prevalence of anemia in different geographic groups with different socioeconomic status (SES), all subgroups were further divided into eight categories (21): Group I (large coastal city), Group II (upper class/large city), Group III (middle class/city), Group IV (lower class/city), Group V (upper class/rural), Group VI (middle class/rural), Group VII (lower class/rural), and Group VIII (western/lower class/rural). Group I included the nine largest cities (Beijing, Shanghai, Tianjin, Shijiazhuang, Shenyang, Dalian, Jinan, Qingdao, and Nanjing) and Group II represented the upper urban class. Group VIII constituted the other extreme: rural regions in western provinces, home to the lowest SES class (data was shown in **Figure 1**).

Statistical Analyses

The prevalence of anemia in the Han children was described by sex, age, and geographic groups. Chi-square test was used to assess the difference of the prevalence and categories of anemia among different subgroups, while the inspection level was adjusted by Bonferroni method [$\alpha' = 2\alpha/k(k-1)$, $k = 7$]. To assess the geographic difference of association between anemia and stunting, binary logistic regression analyses were conducted with adjustments for sex and age. The design effect of cluster sampling by school was also added in the model. All analyses were conducted using Stata 12.1 (Stata Corp, College Station, Texas). A 2-sided P-value of <0.05 was considered significant.

RESULTS

The Prevalence of Anemia in Different Geographic Groups

As shown in **Table S1**, the prevalence of anemia was significantly higher in girls (10.8%) than boys (7.0%). The highest anemia prevalence was in Group VII (12.0%). The lowest prevalence of anemia was observed in Group I (5.2%), Group V (6.0%), and Group II (7.6%). The prevalence of anemia in Group I was more than twice that of Group VII. **Table S2** demonstrates that the moderate/severe prevalence of anemia was concentrated in Group VII and Group VIII for both sexes. The prevalence of anemia at each age group was similar to the total sample and the highest prevalence of anemia was found in the age 7 group, followed by 12 years, and the lowest prevalence was the 14-year-old boys group. For girls, those aged 12 and 14 years had significantly higher anemia prevalence than those of 7 and 9 years (data was shown in **Table S3**).

The Prevalence of Anemia in Susceptible Population

Girls had a higher prevalence of anemia than boys across different groups. The prevalence of anemia among girls in Group VII was 4–5 times higher than boys in Group I (**Table S1**). **Table 1** and **Table S4** indicate that the prevalence of anemia among stunting children was higher than their non-stunting counterparts. When stratified by sex, the prevalence of anemia was higher in stunting boys than non-stunting boys in Group IV ($\chi^2 = 12.78$, $P = 0.002$) and Group VII ($\psi^2 = 6.21$, $P = 0.018$), while for girls, the anemia prevalence was higher in stunting girls than their non-stunting peers only in Group II ($\chi^2 = 4.57$, $P = 0.046$).

The Association Between Anemia and Stunting Stratified by Geographic Groups and Sex

As shown in **Table 2**, the stunting children have a 30% higher risk of anemia than non-stunting children (OR = 1.30, 95% CI: 1.05–1.60). When stratified by geographic group, the association strengths ranged from 1.41 to 2.34 in Group II, III, IV, VI, and

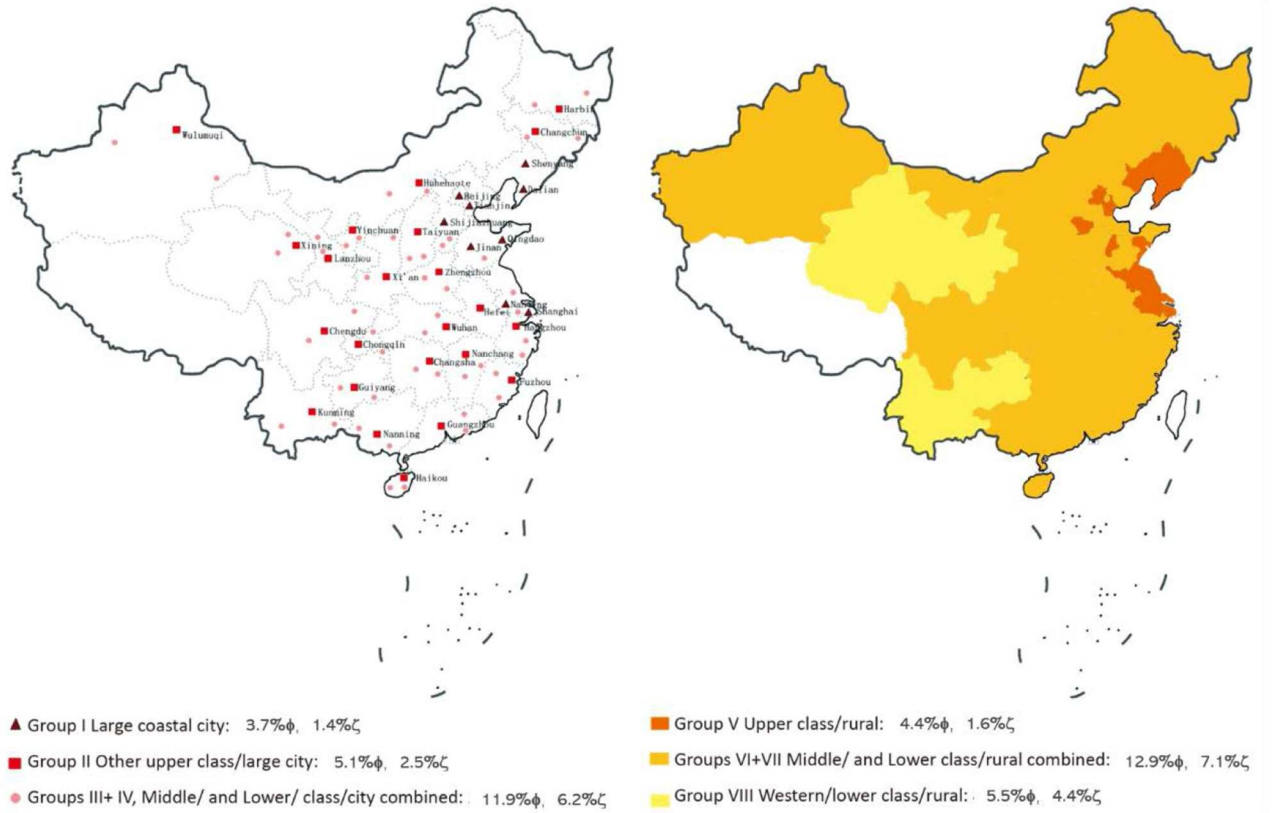

FIGURE 1 | The distribution of different geographic groups with different socioeconomic status (SES); note: ϕ the prevalence of mild anemia in 2014, ζ the prevalence of moderate/severe anemia in 2014.

VIII but with 95% CI overlap, while there was no significant association between anemia and stunting in Group I, V, and VII. After stratifying by sex, stunting boys were 1.74 times more likely to be anemic than non-stunting boys while there was no significant association between stunting and anemia among girls. The results of the associations by age stratification were consistent with total sample (data not shown).

DISCUSSION

In the present study, by using data from the 2014 CNSSCH covering 26 provinces and four municipalities in China, not only were the differences in the prevalence of anemia among school-aged children by geographic groups, sex, and stunting status documented, but also the major associations between anemia and stunting in school-aged children. On the whole, the prevalence of anemia was significantly higher in girls than in boys, and in addition there was low prevalence of anemia in areas with good economic conditions. However, some regions, such as the poorer rural setting of Group VII and middle-class cities of Group III might be overlooked.

Researchers have pointed out that geographic disparity was related inversely to SES, to wealth at a household level, and to income and education at an individual level (2, 22, 23). Areas with SES were often geographically good, and also had better health care facilities and services (24). Compared to rural areas, individuals living in better SES have a greater availability of food and better housing, electricity, piped water, sanitation, and transportation. Moreover, these populations usually have a higher educational level, economic status, and employment opportunities. Studies in Indonesia have found child stunting to be associated with poor health care practices, inadequate sanitation and water supply, food insecurity, and low caregiver education (25). Sankar Goswmai's study used the wealth index to reflect the state of SES and the results indicated that, relatively, the poorest area had the highest prevalence of anemia (OR = 2.033, 95% CI:1.71–2.22), while the richer area had lowest prevalence of anemia (OR = 1.183, 95% CI:1.14–1.32) (26). In groups with these good economic development indicators, the prevalence of anemia was low; the study mentioned that a child living in a household in the lowest wealth quintile was 21% more likely to be anemic than were those in the highest wealth quintile (27), which is consistent with the present study. In Group I (large coastal city) and Group II (other upper class/large city), the economic development is relatively high and anemia prevalence is relatively lower than other groups. High quality living environments and facilities due to advanced economic development can contribute to anemia reduction among Chinese

TABLE 1 | The prevalence of anemia stratified by stunting status, geographic group, and sex among Chinese school-aged children.

Group[#]	Boys						Girls					
	Non-stunting		Stunting		χ^2	*P*	Non-stunting		Stunting		χ^2	*P*
	N	%	*N*	%			*N*	%	*N*	%		
I	98	3.3	1	5.0	13.39	0.066	207	6.9	0	0.0	0.67	1.000
II	299	6.6	4	14.8	2.92	0.100	388	8.5	6	19.4	4.57	0.046
III	374	7.6	8	13.8	3.06	0.084	619	12.7	13	18.6	2.10	0.150
IV	355	6.8	12	17.9	12.78	0.002	454	8.6	10	14.3	2.80	0.129
V	118	4.0	0	0.0	0.29	1.000	240	8.0	1	11.1	0.11	0.531
VI	295	6.7	8	8.6	0.51	0.408	397	9.1	15	15.0	4.04	0.053
VII	710	9.0	15	6.9	1.05	0.395	1201	15.2	29	12.0	1.92	0.202
VIII	161	8.1	22	13.8	6.21	0.018	225	11.3	19	11.6	0.01	0.898
Total	2,410	6.9	70	11.1	16.89	<0.001	3731	10.7	93	13.4	5.11	<0.001

[#]*Group I (large coastal city), Group II (upper class/large city), Group III (middle class/city), Group IV (lower class/city), Group V (upper class/rural), Group VI (middle class/rural), Group VII (lower class/rural), and Group VIII (western/lower class/rural). Group I included the nine largest cities (Beijing, Shanghai, Tianjin, Shijiazhuang, Shenyang, Dalian, Jinan, Qingdao and Nanjing) and Group II, represented the upper urban class. Group VIII constituted the other extreme: rural regions in western provinces, home to the lowest SES class.*

TABLE 2 | Association between anemia and stunting stratified by geographic group and sex among Chinese school-aged children.

Group[#]	Total[a]	Boys[b]	Girls[b]
I	1.17 (0.11–12.63)	29.20 (1.81–471.90)*	-
II	2.32 (1.24–4.36)*	2.78 (1.15–6.71)*	2.17 (0.85–5.55)
III	1.76 (1.12–2.77)*	2.13 (1.14–4.01)*	1.48 (0.88–2.50)
IV	2.34 (1.33–4.12)*	3.51 (1.72–7.15)*	1.72 (0.80–3.70)
V	0.99 (0.14–6.74)	-	1.38 (0.20–9.66)
VI	1.78 (1.16–2.72)*	1.89 (0.87–4.10)	1.79 (1.04–3.07)*
VII	0.77 (0.58–1.02)	0.86 (0.49–1.52)	0.73 (0.51–1.06)
VIII	1.41 (1.00–1.99)*	2.05 (1.30–3.22)*	1.03 (0.62–1.72)
Total	1.30 (1.05–1.60)[a*]	1.74 (1.30– 2.35)[a*]	1.09 (0.86–1.38)[b]

[a]*Adjusted for age, sex, and group.*

[b]*Adjusted for age and group.*

**Groups were significantly different by multivariate logistic regression analysis, $P < 0.05$. (-):the sample size was too small to display the statistics results.*

[#]*Group I (large coastal city), Group II (upper class/large city), Group III (middle class/city), Group IV (lower class/city), Group V (upper class/rural), Group VI (middle class/rural), Group VII (lower class/rural), and Group VIII (western/lower class/rural). Group I included the nine largest cities (Beijing, Shanghai, Tianjin, Shijiazhuang, Shenyang, Dalian, Jinan, Qingdao and Nanjing) and Group II, represented the upper urban class. Group VIII constituted the other extreme: rural regions in western provinces, home to the lowest SES class.*

children (2). A previous study conducted in 32 selected low-income and middle-income countries showed that a child living in a household in the lowest wealth quintile was 21% more likely to be anemic than those in the highest wealth quintile (2). However, the prevalence of anemia did not disappear in Group I and Group II, which indicated that improving the monitoring of anemia, scaling up coverage of prevention, and developing anemia prevention interventions were still needed in China (28, 29).

China has long been concerned about the imbalance of geographic disparity, thus, improving the health of children in rural western China remains a critical health priority (30). The national anemia prevention intervention policy was mainly implemented in the area of Group VIII, i.e., "National Nutrition Improvement Program for Rural Compulsory Education Students (NNIPRCES)" released by the general office of the State Council of China (2011) (31). Even though the prevalence of anemia in Group VIII has been significantly reduced after the implementation of the above policy (32), it is still serious due to its high baseline. Currently, Group VII has replaced Group VIII as the most serious burden of anemia because no extra attention has been given to this group. Other than continuing investment in anemia improvement in rural regions of western provinces, it is recommended that attention should be paid to other areas such as poorer rural settings in China. For example, nutritional education and improvement of diet quality should be included in the content of anemia intervention, to ensure the daily Recommended Dietary Allowance (RDA) of iron-containing foods (meat, milk, etc.) (33). These additional measures may play a more important role in decreasing anemia for vulnerable groups such as children living in western regions, boys with stunting issues, and adolescent girls (14).

Besides Group VII, Group III also had a surprisingly high prevalence of anemia. The higher prevalence of anemia in Group VII indicated that lower economic groups are susceptible and intervention needs to be focused here first, and as such it might be helpful to improve the anemic status by eliminating the geographic disparity. Considering the serious disparity, the government should reconsider the priorities around the anemic burden across the country and develop new strategies and interventions not only to the groups with serious burden, but also those at high risk like Group III.

In the present study, the association between anemia and stunting exists only in boys, but not in girls. Therefore,

non-targeted anemia interventions, such as general nutrition improvement, may only work for boys. Ramachandran (34) pointed out that adolescent girls form a crucial segment of the population and constitute the vital "bridge" between the present generation and the next generation. The main reasons for this consequence among girls may be the menstrual bleeding during their puberty and weight loss (35, 36). In order to pursue a slim figure, adolescent girls deliberately lose their body weight by dieting, which may lead to insufficient iron intake (37). Iron deficiency may be a routine consequence of growth and skeletal development (38). Sex differences should be taken into account when implementing anemia interventions. Consequently, it is suggested that all children, especially adolescent girls, need health education due to their vulnerability to anemia (39). Furthermore, reliable measures on the causes of anemia are needed to guide interventions (40).

China, with its significant economic development in recent decades, has experienced epidemiological and demographic transitions which have affected its population's nutritional conditions and produced environments that have contributed to a change in anemia (41). Despite the nutritional transition and improved nutritional status, stunting and anemia remain a major public health problem in China (42). There were some common factors in the occurrence of anemia and stunting. As we all know, the main risk factor for anemia among children is low iron intake at a stage of life in which iron requirement is high. Iron, as an essential trace mineral, is necessary for linear growth and body tissue proliferation (43). Meanwhile, stunting is an indicator of long-term chronic malnutrition, which is primarily caused by insufficient nutrition supply (44). The supply of nutrients includes not only macro-nutrients, but also micronutrients, such as iron (45). One reason why stunting may be related to anemia is that stunting may pick up deficiencies in iron, which are also known to boost the risk of anemia through impaired erythropoiesis and oxidative stress pathways (44). Especially for children in groups with high prevalence of stunting, all age-groups of children were vulnerable groups for anemia (46). In addition, it is worth noting that school-aged children in Group III (middle class/city) in China have been facing the double burden of anemia and stunting. Therefore, "the child- and adolescent- specific dietary guidance" is able to guide children to a reasonable quality diet, which refers to the "recommended daily dietary allowances for children and adolescents" established by the Chinese Nutrition Society (47). WHO recommends that a diet containing adequate amounts of bioavailable iron could prevent and control anemia (48). In addition, community-based platforms for nutrition education and government commitment and focus on equity are also important factors that may lead to the implementation of interventions that prevent and treat child stunting (49–51).

There are limitations in the present study. Firstly, it was a cross-sectional study and it cannot infer causality between anemia and stunting. Secondly, samples of capillary blood from the fingertip of each child were collected after discarding the first drop. Capillary blood can't distinguish the type and cause of anemia. Thirdly, iron deficiency and other nutrient indicators were not assessed directly.

CONCLUSIONS

The present study demonstrated a large geographic disparity in anemia in China. Lower anemia is found in better SES groups, while the higher prevalence is shown in poorer SES groups. The prevalence of anemia in Group VIII was not only due to SES, but also stunting. In addition to focusing on the rural western regions, the government should also pay more attention and provide more resources to the population in middle class cities and lower-class rural areas. The present findings imply that previous measures aimed at improving anemia, regardless of sex and with a limited focus on school-aged children in poor groups, may not be comprehensive enough to tackle the anemia problem in China. Specific strategies and interventions should be developed for children in susceptible groups, and especially for girls.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The project was approved by the Medical Research Ethics Committee of Peking University Health Science Center (IRB00001052-18002).

AUTHOR CONTRIBUTIONS

J-YW and YS conceptualized and designed the study and completed the statistical analyses. J-YW drafted the initial manuscript and reviewed and revised the manuscript. YS and YM designed the study and collected the data. D-ML and YS assisted with the statistical analyses. YM, JM, PL, BD, P-JH, D-ML, JD, and YS critically reviewed and revised the manuscript. All authors were involved in writing the paper and had final approval of the submitted and published versions.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2020.00193/full#supplementary-material

REFERENCES

1. Stevens GA, Finucane MM, De-Regil LM, Paciorek CJ, Flaxman SR, Branca F, et al. Global, regional, and national trends in haemoglobin concentration and prevalence of total and severe anaemia in children and pregnant and non-pregnant women for 1995–2011: a systematic analysis of population-representative data. *Lancet Global Health.* (2013) 1:e16–25. doi: 10.1016/S2214-109X(13)70001-9
2. Balarajan Y, Ramakrishnan U, Ozaltin E, Shankar AH, Subramanian SV. Anaemia in low-income and middle-income countries. *Lancet.* (2011) 378:2123–35. doi: 10.1016/S0140-6736(10)62304-5
3. Antwi-Bafour S, Hammond S, Adjei JK, Kyeremeh R, Martin-Odoom A, Ekem I. A case-control study of prevalence of anemia among patients with type 2 diabetes. *J Med Case Rep.* (2016) 10:110. doi: 10.1186/s13256-016-0889-4
4. Kojima S. Why is the incidence of aplastic anemia higher in Asia? *Exp Rev Hematol.* (2017) 10:277–79. doi: 10.1080/17474086.2017.1302797
5. WHO. *Worldwide Prevalence of Anaemia 1993-2005 - WHO Global Database on Anaemia*. Geneva: World Health Organization (2008). Available online at: http://www.who.int/iris/handle/10665/43894 (accessed May 2, 2019).
6. Azzopardi PS, Hearps SJC, Francis KL, Kennedy EC, Mokdad AH, Kassebaum NJ, et al. Progress in adolescent health and wellbeing: tracking 12 headline indicators for 195 countries and territories, 1990–2016. *Lancet.* (2019) 393:1101–18. doi: 10.1016/S0140-6736(18)32427-9
7. Cy J. *Modern Child and Adolescent Health*. 2nd ed. Beijing: People Health Press (2010).
8. China NBoSo. *Tabulation on the 2010 Population Census of the People's Republic of China*. (2011). Available online at: http://www.stats.gov.cn/tjsj/pcsj/rkpc/6rp/indexch.htm (accessed May 5, 2019).
9. Zhan Y, Chen R, Zheng W, Guo C, Lu L, Ji X, et al. Association between serum magnesium and anemia: china health and nutrition survey. *Biol Trace Elem Res.* (2014) 159:39–45. doi: 10.1007/s12011-014-9967-x
10. Gan QL, Chen J, Yang T, Li L, Xu P, Pan H, et al. Prevalence of anemia among students from National Improvement Program for Rural Compulsory Students in 2013. *Chin J Sch Health.* (2016) 37:674–75. doi: 10.16835/j.cnki.1000-9817.2016.05.011
11. Luo R K-WM, Rozelle S, Zhang L, Liu C, Sharbono B, Shi Y, et al. Anemia in Rural China's elementary schools- prevalence and correlates in Shaanxi Province's Poor Counties. *Ecol Food Nutr.* (2010) 49:357–72. doi: 10.1080/03670244.2010.507437
12. Iannotti L, Delnatus JR, John ARO, Eaton JC, Griggs JJ, Brown S. Determinants of anemia and haemoglobin concentration in haitian school-aged children. *PLoS ONE.* (2015) 93:1092–98. doi: 10.4269/ajtmh.15-0073
13. Oliveira D, Ferreira FS, Atouguia J, Fortes F, Guerra A, Centeno-Lima S. Infection by intestinal parasites, stunting and anemia in school-aged children from Southern Angola. *PLoS ONE.* (2015) 10:e0137327. doi: 10.1371/journal.pone.0137327
14. Song Y, Wang HJ, Dong B, Wang Z, Ma J, Agardh A. National trends in haemoglobin concentration and prevalence of anemia among Chinese School-Aged Children, 1995-2010. *J Pediatr.* (2017) 183:164–69.e2. doi: 10.1016/j.jpeds.2017.01.012
15. Organization WH. *WHO Reference 2007 SPSS Macro Package*. (2007). Available online at: http://www.who.int/entity/growthref/tools/readme_spss.pdf (accessed May 4, 2019).
16. Onis MD, Onyango AW, Borghi E, Siyam A, Nishida C, Siekmann J. Development of a WHO growth reference for school-aged children and adolescents. *Bull World Health Org.* (2007) 85:660–7. doi: 10.2471/BLT.07.043497
17. Nkrumah B, Nguah SB, Sarpong N, Dekker D, Idriss A, May J, et al. Haemoglobin estimation by the HemoCue(R) portable haemoglobin photometer in a resource poor setting. *BMC Clin Pathol.* (2011) 11:5. doi: 10.1186/1472-6890-11-5
18. World Health Organization (WHO). *Haemoglobin Concentrations for the Diagnosis of Anaemia and Assessment of Severity*. (2011). Available online at: http://apps.who.int/iris/bitstream/10665/85839/3/WHO_NMH_NHD_MNM_11.1_eng.pdf (accessed May 4, 2019).
19. World Health Organization (WHO) U, UNU. *Iron Deficiency Anaemia Assessment, Prevention, and Control, A Guide for Programme Managers*. Geneva: World Health Organization (2001).
20. Chan M. *Haemoglobin Concentrations for the Diagnosis of Anaemia and Assessment of Severity*. China: World Health Organization (2011). p. 1–6.
21. Ji CY, Chen TJ, Working Group on Obesity in C. Empirical changes in the prevalence of overweight and obesity among Chinese students from 1985 to 2010 and corresponding preventive strategies. *Biomed Environ Sci.* (2013) 26:1–12.
22. Melku M, Addis Z, Alem M, Enawgaw B. Prevalence and predictors of maternal anemia during pregnancy in Gondar, Northwest Ethiopia: an Institutional Based Cross-Sectional Study. *Anemia.* (2014) 2014:108593. doi: 10.1155/2014/108593
23. Adamu AL, Crampin A, Kayuni N, Amberbir A, Koole O, Phiri A, et al. Prevalence and risk factors for anemia severity and type in Malawian men and women: urban and rural differences. *Popul Health Metr.* (2017) 15:12. doi: 10.1186/s12963-017-0128-2
24. Alexis J Comber CB, Robert Radburn. A spatial analysis of variations in health access- linking geography, socio-economic status and access perceptions. *Int J Health Geogr.* (2011) 10:1–11. doi: 10.1186/1476-072X-10-44
25. Beal T, Tumilowicz A, Sutrisna A, Izwardy D, Neufeld LM. A review of child stunting determinants in Indonesia. *Matern Child Nutr.* (2018) 14:e12617. doi: 10.1111/mcn.12617
26. World Health Organization (WHO). *Reducing Stunting in Children- Equity Considerations for Achieving the Global Targets 2025*. (2018). Available online at: https://apps.who.int/iris/handle/10665/260202 (accessed May 11, 2019).
27. Goswmai S, Das KK. Socio-economic and demographic determinants of childhood anemia. *J Pediatr.* (2015) 91:471–7. doi: 10.1016/j.jped.2014.09.009
28. Okumura MJ, Knauer HA, Calvin KE, Takayama JI. Caring for children with special health care needs: profiling pediatricians and their health care resources. *Matern Child Health J.* (2018) 22:1042–50. doi: 10.1007/s10995-018-2484-3
29. Dreshaj HNA. Global challenges for the environment, water clean and economic advantages. *Int J Eng Trends Technol.* (2013) 4:1–5. Available online at: https://ssrn.com/abstract=2835964
30. Huo YJ, Zhou H, Wang Y. Status of underweight and it's relative factors for children under 3 years old in 14 counties in the western areas of China. *Chin Journal Child Health Care.* (2017) 25:552−5. doi: 10.11852/zgetbjzz2017-25-06-04
31. Cardoso MA, Augusto RA, Bortolini GA, Oliveira CS, Tietzman DC, Sequeira LA, et al. Effect of providing multiple micronutrients in powder through primary healthcare on anemia in young Brazilian children: a multicentre pragmatic controlled trial. *PLoS ONE.* (2016) 11:e0151097. doi: 10.1371/journal.pone.0151097
32. Aonsfsl WT. *People's Republic of China: Health Industry Standard* (China). (1998).
33. de Souza Queiroz S, de A. Torres M. Iron deficiency anemia in children. *J Pediatr.* (2000) 76:298–304. doi: 10.2223/JPED.167
34. Ramachandran P. Health and nutrition in adolescents and young women: preparing for the next generation. *J Health Med Nurs.* (2017) 145:256–57. doi: 10.1016/j.ijhydene.2013.08.087
35. Choi JLMSS. Attempts to lose weight among US children- importance of weight perceptions from self, parents, and health professionals. *Obesity.* (2018) 26:597–605. doi: 10.1002/oby.22106
36. Powers J M, Stanek J R, Lakshmi S, Haamid FW, O'Brien SH. Hematologic considerations and management of adolescent girls with heavy menstrual bleeding and anemia in U.S. Children's Hospitals. *J Pediatr Adolesc Gynecol.* (2018) 31:446–50. doi: 10.1016/j.jpag.2018.06.008
37. Zhang CX, Chen YM, Chen WQ, Su YX, Wang CL, Wu JN. Food group intake among adolescents in guangzhou city compared with the Chinese Dietary Guidelines. *Asia Pac J Clin Nutr.* (2012) 21:450–6. doi: 10.6133/apjcn.2012.21.3.18
38. Verma A, Rawal VS, Kedia G, Kumar D, Chauhan J. Factors influencing anemia among girls of school going age (6–18 years) from the slums of Ahmedabad City. *Ind J Comm Med.* (2004) 29:25−6.
39. Thummakomma K, Meda P, Jayashankar T. Impact of change in dietary behavior and iron supplemention for reduction of iron

deficiency anemia in rural adolescent girls. *Int J f Agric.* (2017) 7:525–28. doi: 10.24247/ijasraug201768
40. Patton GC, Sawyer SM, Santelli JS, Ross DA, Afifi R, Allen NB, et al. Our future: a Lancet commission on adolescent health and wellbeing. *Lancet.* (2016) 387:2423–78. doi: 10.1016/S0140-6736(16)00579-1
41. Mikki N, Abdul-Rahim HF, Awartani F, Holmboe-Ottesen G. Prevalence and sociodemographic correlates of stunting, underweight, and overweight among Palestinian school adolescents (13-15 years) in two major governorates in the West Bank. *BMC Public Health.* (2009) 9:485. doi: 10.1186/1471-2458-9-485
42. Zou Y, Zhang R-H, Xia S-C, Huang LC, Fang YQ, Meng J, et al. The rural-urban difference in BMI and anemia among children and adolescents. *Int J Environ Res Public Health.* (2016) 13:1020. doi: 10.3390/ijerph13101020
43. Bruce BR. Modern nutrition in health and disease (tenth edition). *Crit Care Med.* (2006) 34:529–32. doi: 10.1097/01.CCM.0000236502.51400.9F
44. Fatimah APS. Iron intake and haemoglobin levels in stunting in adolescent. *Althea Med J.* (2016) 3:175–80. doi: 10.15850/amj.v3n2.782
45. Ramakrishnan U, Nguyen P, Martorell R. Effects of micronutrients on growth of children under 5 y of age: meta-analyses of single and multiple nutrient interventions. *Am J Clin Nutr.* (2008) 89:191–203. doi: 10.3945/ajcn.2008.26862
46. Assefa S, Mossie A, Hamza L. Prevalence and severity of anemia among school children in Jimma Town, Southwest Ethiopia. *BMC Hematol.* (2014) 14:1–9. doi: 10.1186/2052-1839-14-3
47. Ge KY. *Chinese Nutrition Scientific Encyclopedia*. Beijing: People's Medical Publishing House (2004). p. 1521–44.
48. World Health Organization (WHO). *Global Nutrition Targets 2025: Anaemia Policy Brief.* (2014). Available online at: https://www.who.int/nutrition/publications/ (accessed June 22, 2019)
49. Smith LC KF, Frankenberger TR, Wadud A. Admissible evidence in the court of development evaluation? The Impact of CARE's SHOUHARDO project on child stunting in Bangladesh. *IDS Working Papers.* (2011) 41:196–216. doi: 10.1111/j.2040-0209.2011.00376_2.x
50. Bhutta ZA, Das JK, Rizvi A, Gaffey MF, Walker N, Horton S, et al. Evidence-based interventions for improvement of maternal and child nutrition: what can be done and at what cost? *Lancet.* (2013) 382:452–77. doi: 10.1016/S0140-6736(13)60996-4
51. Al Hassand N. The prevalence of iron deficiency anemia in a Saudi University female students. *J Microsc Ultrastruct.* (2015) 3:25. doi: 10.1016/j.jmau.2014.11.003

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Differential Methylation in Promoter Regions of the Genes *NR3C1* and *HSP90AA1,* Involved in the Regulation, and Bioavailability of Cortisol in Leukocytes of Women With Preeclampsia

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Introduction: Hypertensive disorders are of interest in obstetrics and gynecology because they are the second place among causes of maternal mortality and a source of complications in the short, mid, and long term. Even if the pathophysiological process behind preeclampsia (PE) is still unknown, stress factors have been revealed to play an important role in the genesis of this pathologic process.

Methods: A case-control study was designed with the purpose of determining if there is a differential methylation in *NR3C1*, *HSD11B2*, *CYP11A1*, *CRHBP*, *TEAD3*, and *HSP90AA1* genes, related to signaling of the hypothalamic–pituitary–adrenal axis, and its regulation on early-onset PE (EOPE).

Results: A total of 20 cases and 20 controls were studied by DNA methylation analysis, demonstrating differences among groups in the percentage of methylation of the *NR3C1* gene. After a contingency analysis, an odds ratio (OR) for PE of 12.25 was identified for *NR3C1* and 9.9 for *HSP90AA1* genes. *NR3C1*, *TEAD3*, and *HSP90AA1* genes showed a positive correlation with the systolic and diastolic blood pressure levels with a $p \leq 0.05$.

Conclusion: This study found a differential methylation in the glucocorticoid receptor (GR) *NR3C1* and its co-chaperone *HSP90AA1* in women with PE, with a possible regulatory role in the response to stress in pregnancy and is a likely physiopathological mechanism in PE.

Keywords: preeclampsia, pregnancy, methylation, cortisol, hypothalamic–pituitary–adrenal axis

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INTRODUCTION

Preeclampsia (PE) is a multisystemic syndrome of pregnancy and the puerperium, with a reduction of systemic perfusion, generated by vasospasm and the activation of coagulation systems (1). Approximately 10–15% of maternity deaths are directly associated with PE, with an estimated toll of 75,000 maternal deaths and 500,000 neonatal deaths per year. In Mexico, it constitutes the main cause of death associated with complications during pregnancy and represents 34% of the total maternal mortality (2).

It has been proven that the risk of developing PE is related to the maternal psychosocial status during pregnancy, requiring the evaluation of family and marital and social conditions. A disproportionate response to stress results in a metabolic derailment in the regulation by the hypothalamic–pituitary–adrenal (HPA) axis (3). Likewise, maternal stress and/or a rise of glutocorticoids *in utero* is associated with low birth weight and metabolic disorders among the offsping, in adulthood (4). PE is a syndrome of idiopathic origin that can present manifestations in practically all apparatuses and systems of the fetus–maternal binomial. The causal framework of PE is still poorly understood, but the literature is inclined to explain it through the deficient processes of placentation (5).

Various studies have documented an association between adverse cultural and psychosocial situations with a high frequency of gestational morbimortality (6, 7). Research-based approaches establish a clear association between a context characterized by high social stressors and low psychosocial support with the expression of PE. Studies in the area of psychoneuroimmunoendocrinology have shown that individuals exposed to chronic stress, anxiety, and/or other factors undergo immunologic disorders, suggesting that stress and emotions interfere with immunoregulatory mechanisms (8). Glucocorticoids produced in the adrenal cortex, in response to the signals from the HPA axis, can affect different cellular functions during pregnancy. It has also been reported that in pregnant women with PE, a premature rise of placental cortisol, joined by a reduced activity of the enzyme 11-beta-hydroxyesteroid dehydrogenase type 2 (HSD11B2), transforms cortisol into cortisone. Glucocorticoids also inhibit the luteinizing hormone (LH), estrogens, progesterone, and the ovarian steroid hormones. Significantly, a high activity of glucocorticoids established by an increase in the levels of cortisol in circulation or the altered metabolism of cortisol can lead to a resistance to insulin, closely associated with hypertension and endothelial resistance—characteristics observed along with PE (9).

The association between PE and epigenetic alterations of the placenta (6–8) has been recently reported. The maternal epigenome has a unique history that can be useful to study in order to understand its susceptibility to develop PE, as well as to detect it in its early stages (10). Contrary to the genotype (non-modifiable), epigenetics can confer a degree of phenotypic plasticity linked to stress, nutrition, or any other change in the environment, allowing the fetus to respond to the environment and making changes in the genetic expresion in accordance to this. Furthermore, if the epigenetic alterations occur in the gametes, these can be inherited and can bring phenotypic consequences to the next generation; for example, the possibility of epigenetic disorders previous to conception or during pregnancy can increase the susceptibility to PE (10).

A study describes a different pathophysiological behavior in early-onset PE (EOPE) and in late-onset PE (LOPE). They informed, in a case-control study, that in EOPE, a differential percentage of methylated DNA in CpG sites in genes encode for the receptor of glucocorticoids (*NR3C1*) and binding protein to CRH (*CRHBP*) (11). In othes studies are described the association between the degree of the methylation of the promoters of the gene *NR3C1*, with the presence of normotension, hypotension, and hypertension in a group of pregnant women, and how these can be found present independently from confounding variables such as tobacco use, family history of High Blood Pressure (HBP), or body mass index (BMI) (12, 13).

The purpose of this work was to determine if there is a differential methylation in *NR3C1*, *HSD11B2*, *CYP11A1*, *CRHBP*, *TEAD3*, and *HSP90AA1* genes related to signaling of the HPA axis and its regulation on EOPE.

MATERIALS AND METHODS

Subjects

The present study was revised and approved by the Commitee of Ethics in Research of the General Hospital of Durango with registration number 456/015.

A paired case-control study was designed. The cases were pospartum women with (1) EOPE diagnosis, (2) pregnancy between 20 and 34 weeks of gestation, (3) age between 18 and 45, and (4) residents of the State of Durango, México. The participants in the control group were pospartum women without any complications. The groups were paired individually by age, place of residence, and pregnancy stage.

The size of the sample was calculated with the aid of SigmaPlot software, considering a minimal methylation difference to detect 12% among the study groups, a power of 95%, and an α value of 0.05, resulting in the groups composed of 20 cases and 20 controls.

Patients excluded from the study were all women with comorbidities (hypertension, diabetes, rheumatoid arthritis, systemic lupus erythematosus, psychiatric illness, antiphospholipid syndrome, Down syndrome, ischemic cardiopathy, kidney diseases) and women who presented PE in previous pregnancies, multiple pregnancies, hydatidiform mole, and miscarriages.

We used a case report format for the recollection of sociodemographic data. The American College of Obstetricians and Gynecologists 2013 criteria for the diagnosis of PE were considered, which include (1) systolic blood pressure $\geq$140 mmHg and/or diastolic blood pressure $\geq$90 mmHg, (2) pregnancy of more than 20 weeks of gestation, and (3) $\geq$300 mg proteinuria in 24-h urine or $\geq$1+ in test strip (14).

Measures and Samples

Blood pressure was determined by an OMRONHEM7121 sphygmomanometer, the gestation week was inferred by ultrasound and proteinuria by urine analyses. The 24-h urine

sample was used for the determination of proteinuria; the sample was collected into a sterile vessel. In a maximum period of 24 h postpartum, peripheral blood samples (BD Vacutainer® EDTA 4 ml) were collected from each participant. All the samples were frozen until the moment of their analysis, for DNA purification utilizing the standardized procedure described by Iranpur et al. (15). The quantification of DNA (A 260 nm), as well as the determination of purity (A 260/280), was conducted through spectrophotometry in a DU-730 Uv-Vis Bekman Coulter spectrophotometer. The DNA samples were stored at −70°C until processing in methylation assays.

DNA Methylation Analysis

An *in silico* study was carried out utilizing the online Eukaryotic Promoter Data Base (EDP) software to locate the CpG Islands in/or near the transcription start site of candidate genes (16). The methylation analysis was conucted *via* qPCR employing reagents EpiTectMethyl II qPCR Primer Assay and EpiTect II DNA Methylation Enzime Kit (Qiagen®) in accordance with the instructions of the manufacturer. The determination of the percentage of methylation with these reagents is based upon the digestion of the restriction sites with methylation-sensitive enzymes. The genomic coordinates of the analyzed CpG islands are presented in the **Supplementary Table 1**. The reactions of qPCR in real time were conducted in a thermal cycler ECO Illumina® (San Diego, CA) using the v.1 software. To transform the Ct values obtained from the reaction of a PCR to percentages of methylation, a spreadsheet provided by Qiagen® was employed.

Statistical Analyses

The data were analyzed with the statistical program SPSS v.21, central tendency and dispersion measures were calculated. The *T*-test was used to compare means between groups, in cases where medians were compared, the Mann–Withney *U*-test was used. The chi square test was used to compare percentages. An analysis of the correlation of clinical variables with methylation percentage was carried out employing Spearman's rho. The cutoff points for the contingency analysis were performed with the online software Cutoff Finder (17), and the odds ratio (OR) was calculated for each of the genes in the study. A value of $p \leq 0.05$ of statistical significance was considered.

RESULTS

Between October 2015 and October 2016, 830 postpartum women were screened for the study, out of which 112 presented a PE clinical condition, from whom 20 were selected by fulfilling the inclusion criteria. The sociodemographic and clinical variables of the participants are presented in **Tables 1, 2**.

The methylation analysis showed differences among the groups for *NR3C1* gene with a median in the case group of 89.21 (interquartile range 73.3–92.2) vs. 22.1 (0.27–50) in the controls ($p = 0.003$) (**Table 3**). The methylation percentage of the genes *TEAD3*, *HSP90AA1*, *CYP11A1*, *HSD11B2*, and *CRHBP* did not show any statistically significant differences; however, a marginal association can be perceived in the first two, which presented significance in posterior analyses (**Table 3**).

The correlation analysis of clinical variables with the methylation percentage showed a direct proportional relation between the methylation and elevation of blood pressure for genes *NR3C1, TEAD3*, and *HSP90AA1*, particularly for diastolic pressure (**Table 4**).

Finally, a contingency analysis was performed to determine the OR in the presence or absence of PE and the methylation state of each of the genes in the study; finding ORs of 12.25 and 9.9 for *NR3C1* and *HSP90AA1* genes, respectively, both statistically significant. The remaining genes (*CYP11A1*, *HSD11B2*, *CRHBP*) did not present an association with the developmet of PE (**Table 5**).

TABLE 1 | Sociodemographic characteristics from a sample of women diagnosed with preeclampsia and normotensive.

		Cases ($n = 20$)		Controls ($n = 20$)		p
ORIGIN						
	Rural	15	75.0%	15	75.0%	
	Urban	5	25.0%	5	25.0%	1
Occupation	Seamstress	1	5.0%	0	_	
	Teacher	1	5.0%	0	_	
	Housewife	17	85.0%	19	95.0%	
	Office employee	0	_	1	5.0%	
	Student	1	5.0%	0	_	0.391
MARITAL STATUS						
	Single	3	15.0%	7	35.0%	
	Married	6	30.0%	2	10.0%	
	Domestic partnership	11	55.0%	11	55.0%	0.87

TABLE 2 | Clinical information obtained from a sample of preclamptic women vs. normotensive.

	Cases ($n = 20$)		Controls ($n = 20$)		p
Maternal age*	28.45	± 7.6	28.5	± 7.8	0.969
Gestational age**	31	(28–33)	39.3	(38–40)	0.001
SBP**	160	(150–172)	110	(92–120)	0.001
DBP**	105	(91–110)	67.5	(60–77)	0.001
MBP**	122	(112–127)	81.6	(73–89)	0.001
	n	**%**	***n***	**%**	
Sex of baby					
Male	12	60.0%	13	65.0%	
Female	8	40.0%	7	35.0%	0.114
End of pregnancy					
Cesarean section	19	95%	4	20.0%	
Vaginal delivery	1	5%	16	80.0%	0.001
First pregnancy	9	45.0%	3	15.0%	
More than one pregnancy	11	55.0%	17	85.0%	0.011

*SBP, Systolic blood pressure; DBP, Diastolic blood pressure; MBP, Mean blood pressure. *mean and standard deviation. **median and quartiles 25 and 75.*

TABLE 3 | Differential methylation in the CPG islands of gene regulators of hypothalamic-pituitary-adrenal axis.

Gene	Chromosomal location	CpG Island	Mean of % of methylation (q25–q75)				*P*
			Women with preclampsia		Women without preeclampsia		
NR3C1	5q31.3	111,914	89.2	(73.3–97.2)	22.1	(0.27–50)	0.0003*
CYP11A1	6p21.31	112,418	44.0	(0.8–88.4)	3.7	(0.42–68.7)	0.377
TEAD3	16q22.1	105,382	90.0	(50–97.2)	51.8	(1.72–81.6)	0.077
HSD11B2	15q24.1	104,679	60.7	(1.46–95.4)	80.8	(1.73–99.90)	0.446
HSP90AA1	14q32.31	104,327	70.2	(0.40–79.4)	1.3	(0.05–8.4)	0.068
CRHBP	5q13.3	111,616	50.0	(33.12–64.1)	40.0	(0.52–95.2)	0.579

*N = 20 cases and 20 controls. *p < 0.05.*

TABLE 4 | [a]Correlation analisis between gene methylation and blood pressure.

Gene	SBP		DBP	
	Coefficient of correlation	*p*	Coefficient of correlation	*p*
NR3C1	0.547	0.002*	0.562	0.001*
CYP11A1	0.202	0.27	0.19	0.305
TEAD3	0.469	0.05*	0.551	0.018*
HSD11B2	−0.058	0.755	0.071	0.706
HSP90AA1	0.379	0.052	0.386	0.046*
CRHBP	0.131	0.525	0.222	0.275

[a]*The information was analyzed using the Spearman's rank correlation.*
*N = 40. *Statistical significance ≤ 0.05.*
SBP, Systolic blood pressure; DBP, Diastolic blood pressure.

TABLE 5 | Hipermethylation frequency[a] in the genes of study.

	Women with preeclampsia (%)	Women without preeclampsia (%)	x^2	*P*	OR	95% CI
NR3C1	46.7	6.7	6.13	0.013*	12.25	(1.27–118.37)
CYP11A1	56.2	40.0	0.819	0.366	1.92	(0.46–8.05)
HSD11B2	50.0	60.0	0.313	0.576	0.66	(0.16–2.77)
HSP90AA1	64.3	15.4	6.6	0.01*	9.9	(1.54–63.69)
CRHBP	15.4	30.8	0.352	0.867	0.4	(0.06–2.77)

[a]*The cut point was taken as appointed by the cut off finder (18).*
**Statistical significance p < 0.05.*
Differences between cases and controls.

DISCUSSION AND CONCLUSIONS

In this study, differences in the percentage of DNA leukocyte methylation in the study groups were demonstrated, particularly in that which refers to the *NR3C1* gene. This result is congruent with that reported by Hogg et al. (11) who described higher methylation in the placenta of women with EOPE when compared with healthy controls.

With reference to genes *CYP11A1, TEAD3, HSD11B2, HSP90AA1*, and *CRHBP*, when a clustering categorization is executed, we obtain significant differences in *NR3C1* and *HSP90AA1* genes, presenting 12.25 and 9.9 more probability of developing PE in those women in hypermethylation states in comparison with those who presented a lower methylation, with 95% CI that is statistically significant. The results are also consistent with that reported in 2017 by Dwi Putra et al. (12) who found an association between the regulation of blood pressure in pregnant women and the methylation of the promoter of *NR3C1*.

Even though there are no reports in the literature that directly relate methylation of this co-chaperone with PE, Ilona et al. (18) demonstrated an association between the expression of the heat shock protein HSP70 and pregnancy complications. In other HSP proteins, like 27, 60, and 90, no differences were found due to the small size of the sample. Our findigs agree with the theoretical foundation of the function of the co-chaperone HSP90, which promotes the maturation of the glucocorticoid receptor (GR), parting from an appropriate folding state in conformity to where it has a high cortisol affinity.

Only concluding data are presented in the differences found in *NR3C1* and *HSP90AA1*. Although a disparity was observed among the medians of other genes in the study (*CYP11A1*, *TEAD3*), this did not achieve a statistical significance of <0.05; however, we propose that they serve as a reference for future research, probably with a larger study sample size.

The GR exerts its function on the HPA axis, and several studies have demonstrated its expression in a wide diversity of cell types. Therefore, its regulation includes DNA methylation, alternative splicing, and translational mechanisms (19). This indicates that its regulation is very complex, and the expression-specific tissue depends on this process. However, methylation is a mechanism that has proven to be more durable; in this regard, the study reported by Tyrka et al. (20) determined that stressful events can lead to epigenetic changes that remain in leukocytes and this methylation could alter the expression of GR and contribute to the imbalance in homeostasis of the HPA axis. According to the above, methylation specifies whether it could mean a reduction in protein levels and could affect GR expression in target cells.

The results of the present report suggest that the analysis of blood methylation is a potential biomarker due to its easy access and availability at any moment of pregnancy. A marker that distinguishes women with a high or low risk can aid in the selection of patients and offer supervision with a better delivery of the available resources. One of the main challenges within the area of preventive medicine is to find biomarkers

capable of identifying the onset of a disease, especially in a simple non-invasive manner.

The importance of diagnostic and prognosis PE data is due mainly to its close relationship with maternal mortality in our country and the world (156 maternal accumulated deaths in week 11 of 2018 in Mexico, 21.2% associated with pregnancy hypertensive disorders) (21). The possibility of finding potential biomarkers as well as contributing to the comprehension of the articulated pathophysiological mechanisms makes this relevant in the medical arena. An early therapeutic intervention with diverse targets, starting with the justification of using acetylsalicylic acid or low-molecular-weight heparin, as the ACOG guides suggest, and psychotherapeutical interventions for containment and stress management are some examples (18).

In spite of efforts from public health specialists to decrease its impact, in a study of 830 puerperal females, 13% of those explored were diagnosed with PE, suggesting that the problem is prevalent. Within this group, 17% had EOPE, which worsens the matter because according to the American Heart Association, PE before 34 weeks of gestation relates to a higher cardiovascular risk factor than LOPE (18).

In conclusion, this study found a differential methylation in the GR NR3C1 and its co-chaperone HSP90AA1 between preeclamptic and normotensive women, with a possible regulatory role in the response to stress in pregnancy and a possible physiopathological mechanism in PE. These findings contribute to the strengthening of the causal network paradigm previously described by other authors (6, 8, 22–24). Moreover, they support the relation between PE and an unfavorable psychosocial environment, an imbalance in allostatic factors that contribute to a limited placenta formation that can even be present before PE occurence.

STRENGHTS AND LIMITATIONS

One of the strengths of this study is the design and its strict control of the inclusion criteria. Unfortunately, due to these criteria, a large number of women had to be excluded, resulting in a small sample that finally had an impact on the confidence intervals. Considering the relevance of our findings, studies involving a larger sample are suggested for the future, as well as the use of additional technical techniques that show protein expression or gene sequencing.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Commitee of Ethics in Research of the General Hospital of Durango with registration number 456/015. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

QT-S performed experimental and statistical work, wrote the manuscript. MR-R performed the methylation analyses. AS-Á participated in the recruitment of cases. RP-M participated in the interpretation of the results, their discussion and wrote the manuscript. YM-L and JS-M participated in the design and performed of the study, wrote the manuscript and approval of the final version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2020.00206/full#supplementary-material

REFERENCES

1. Gallo M, Vigil-De Gracia P. *Trastornos Hipertensivos del Embarazo*. 1ª ed. España: Editorial AMOLCA (2013).
2. Sánchez E, Nava S, Morán C, Romero J, Cerbón M. Estado actual de la preeclampsia en México: De lo epidemiológico a sus mecanismos moleculares. *Rev Invest Clin*. (2010) 62:252–60.
3. Salvador J, Martínez Y, Lechuga A, Terrones A. Hipertensión inducida por el embarazo en Adolescentes: Un estudio multicéntrico. *Ansiedad y Estrés*. (2005) 11:181–4.
4. Harris A, Seckl J. Glucocorticoids, prenatal stress and the programming of disease. *Horm Behav*. (2011) 59:279:289. doi: 10.1016/j.yhbeh.2010.06.007
5. Sibai B, Dekker G, Kupferminc M. Preeclampsia. *Lancet*. (2005) 365:785–97. doi: 10.1016/S0140-6736(05)17987-2
6. Salvador J. La Dimensión biopsicosocial de la preeclampsia: Un abordaje conceptual empírico. *Ginecol Obstet Mex*. (2009) 77:231–7.
7. Salvador J, Martínez Y, Ramírez J, Aguilar M, Terrones A, Reyes M. Genesis of preeclampsia: an epidemiological approach. *ISRN Obstetr Gynecol*. (2012) 2012:916914. doi: 10.5402/2012/916914
8. Vianna P, Bauer M, Dornfeld D, Bogo J, Distress conditions during pregnancy may lead to pre-eclampsia by increasing cortisol levels and altering

lymphocyte sensitivity to glucocorticoids. *Med Hypotheses.* (2010) 77:188–91. doi: 10.1016/j.mehy.2011.04.007
9. Bertram C, Hanson M. Prenatal programming of posnatal endocrine responses by glucocorticoid. *Reproduction.* (2002) 124:459–67. doi: 10.1530/reprod/124.4.459
10. Choudhury M. Friedman JE. Epigenetics and microRNAs in preeclampsia. *Clin Exp Hypertens.* (2012) 34:334–34. doi: 10.3109/10641963.2011.649931
11. Hogg K, Blair J, McFadden D, Von Dadelszen P, Robinson W. Early Onset Pre-eclampsia is associated with altered DNA methylation of cortisol-signalling and steroidogenic genes in placenta. *PLoS ONE.* (2013) 8:e62969. doi: 10.1371/journal.pone.0062969
12. Dwi Putra SE, Reichetzeder C, Meixner M, Lieve K, Slowinski T, Hocher B. DNA methylation of the glucocorticoid receptor gene promoter in the placenta is associated with blood pressure regulation in human pregnancy. *J Hypert.* (2017) 35:2276–86. doi: 10.1097/HJH.0000000000001450
13. Thailaganathan B, Wormald B, Zanardini C, Sheldon J, Ralph E, Papageorghiou, et al. Early-Pregnancy multiple serum markers and second trimester uterine artery Doppler in predicting preeclampsia. *Obstet Gynecol.* (2010) 115:1233–8. doi: 10.1097/AOG.0b013e3181dd5137
14. American College of Obstetricians and Gynecologists. Hypertension in pregnancy. *Obstet Gynecol.* (2013) 122:1122–31. doi: 10.1097/01.AOG.0000437382.03963.88
15. Iranpur M, Esmailizadeh. *Rapid Extraction of High Quality DNA from Whole Blood Stored at 4° C for Long Period.* Kerman: Department of Animal Science, Faculty of Agriculture, Shahid Bahonar University of Kerman. (2010).
16. Périer R, Praz V, Junier T, Bonnard C, Bucher P. The Eukariotic Promoter Database (EPD). *Nucleic Acids Res.* (2000) 28:302-3. doi: 10.1093/nar/28.1.302
17. Budczies J, Klauschen F, Sinn BV, Gyorffy B, Schmitt WD, Darb-Esfahani S, et al. Cutoff finder: a comprehensive and straightforward web application enabling rapid biomarker cutoff optimization. *PLoS ONE.* (2012) 7:e51862. doi: 10.1371/journal.pone.0051862
18. IIona H, Dvorakova L, Kotlabova K, Kestlerova A, Hympanova L, Novotna V, et al. Assessment of placental and maternal stress responses in patients with pregnancy related complications via monitoring of heat shock protein mRNA levels. *Mol Biol Rep.* (2015) 42:625–37. doi: 10.1007/s11033-014-3808-z
19. Turner J, Muller C. Structure of the glucocorticoid receptor (NRC1) gene 5' untraslated region: identification, and tissue distribution of multiple new human exon 1. *J Mol Endocrinol.* (2005) 35:283.292. doi: 10.1677/jme.1.01822
20. Tyrka AR, Parade SH, Welch ES, Ridout KK, Price LH, Marsit C, et al. Methylation of the leukocyte glucocorticoid receptor gene promoter in adults: associations with early adversity and depressive, anxiety and substance-use disorders. *Transl Psychiatry.* (2016) 6:e848. doi: 10.1038/tp.2016.112
21. Freyermuth G. *Observatorio de Mortalidad Materna.* pág web disponible en (2018). Available online at: http://www.omm.org.mx/index.php, visitado el 11/Abril/2018
22. Monk C, Tianshu F, Lee S, Krupska I, Champagne F, Tycko B. Distress during pregnancy: epigenetic regulation of placenta glucocorticoid-related genes and fetal neurobehavior. *Am J Psychiatry.* (2015) 173:705–13. doi: 10.1176/appi.ajp.2015.15091171
23. Veerbeek J, Hermes W, Breimer A, Rijn B, Koenen S, Mol Ben, et al. Cardiovascular disease risk factors after early-onset preeclampsia, late-onset preeclampsia, and pregnancy-induced hypertension. *Hypertension.* (2015) 65:600–6. doi: 10.1161/HYPERTENSIONAHA.114.04850
24. Giurgescu C, Sanguanklin N, Engeland C, White-Traut R, Park C, Mathews HL, et al. Relationships among psychosocial factors, biomarkers, preeclampsia, and preterm birth in African American women: a pilot. *Appl Nurs Res.* (2015) 28:e1–6. doi: 10.1016/j.apnr.2014.09.002

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ORIGINAL RESEARCH
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Factors Associated With Late Prematurity in the University Hospital of Valle Cali, Colombia During 2013–2014

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Introduction: The birth rate of late premature babies has been increasing in recent years, composing now 75% of all premature births. This growing trend can be explained by different demographic transformations such as an increase in the demand for infertility treatments, older maternal age and the higher incidence of multiple pregnancies, cesarean sections, and labor induction. These premature babies contribute 30% to the global neonatal mortality rate.

Objective: To identify the factors associated with late prematurity at the Hospital Universitario del Valle during the years 2013–2014.

Methodology: Case and control design, 424 patients, 212 cases and 212 controls participated. Cases were defined as newborns with gestational age between 34 and 36 weeks and 6 days old. For the analysis, logistic regression models were developed and association forces (OR) were determined.

Results: A univariate analysis shows that the proportion of teenage pregnant women corresponds to 22.64%. Bivariate analysis shows the maternal morbidity due to hypertensive disorders was 1.6 times higher (95% CI 1.06–2.63), the obstetric alterations in 2.9 times (CI of 95% 1.56–5.44), late preterm infants require more oxygen support 3.26 times (95% CI 1.76–6.03). After adjusting the model, it was found that late premature infants have a 3-fold probability of requiring some resuscitation maneuver (ORa 3.23 95% CI 2.09–4.99), birth is higher by cesarean section by 4.17 times (ORa 4.17 IC 95% 2.50–6.98), maternal morbidity was higher in 1.37 times (ORa 1.37 95% CI 1.14–1.65). The morbidity of the newborn was greater, close to the statistical significance for late premature infants in 1.26 times (ORa 1.26 95% CI 0.97–1.64).

Conclusions: Late premature births in this study show a higher probability of developing morbidity, have a greater opportunity to be born by cesarean section, are products of mothers with morbidity (specifically hypertensive disorders), and require further resuscitation with a need of early obstetric intervention.

Keywords: late premature, associated factors, premature, morbidity, newborn

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INTRODUCTION

The premature delays correspond to about 74% of all premature births and 8% of total births (1). The American Academy of Pediatrics and the American College of Obstetricians and Gynecologists (ACOG) have defined late preterm infants as those born between the 34th and 36th weeks and 6 days of gestation, and have emphasized that these newborns are premature and as such, are at risk of medical complications related to immaturity. They are physiologically and metabolically immature and have a limited compensatory response capacity against extra-uterine changes compared to those born at term, which determines a high risk of morbidity and mortality (2).

As time progresses, care for the newborn evolves. However, late premature babies are today a cause of special interest, since they constitute a large percentage of neonatal mortality (2). Physiological, anatomical and metabolic deficiencies predispose these children to develop complications in the short and long term, with risk of resuscitation at birth twice as large compared to term infants (2).

Respiratory morbidity in late preterm infants has a high prevalence, with pathologies such as respiratory distress syndrome and transient tachypnea of the newborn, which lead to the need for resuscitation at birth, the supply of oxygen. and even ventilatory support (2). The current explanations for this pathologies are:

- Reduction of central chemosensitivity, which makes the body more tolerant of high concentrations of CO_2.
- Immaturity of brain development, which leads to a poor response to physical and metabolic stress.
- Disruption of lung maturation, between the terminal and alveolar sac phase, which causes functional deficiencies of surfactant and inappropriate water management in the lung.

In these babies, the generated systemic failures decrease the functionality of the hypothalamus, hormonal concentrations and fat stores, contributing to instability in body temperature (hypothermia). Similarly, there are alterations in the suction-swallowing-breathing coordination, poor gastrointestinal motility, and low motor tone that produce failures in suction and swallowing processes, poor weight gain, dehydration and increased risk of death (3, 4).

Late preterm infants have an increased risk of developing neurodevelopmental difficulties by 7 years of age, as well as poor performance on standardized tests and developmental delay when compared to term children (1). This is because approximately 50% of the increase in cortical volume occurs between weeks 34 and 40 of gestation, and it is considered that between weeks 36 and 40 there is an exponential increase in gray matter and myelination of white matter. This explains why they present 36% more risk of developmental delay or disability (5, 6).

There is a marked tendency in the literature to develop research focusing mainly on the comparison between late preterm infants and term infants, regarding the complications developed in each group. Various papers have been published on the associated risk factors, such as multiple pregnancies, advanced maternal age, maternal obesity, and premature rupture of membranes (5–7).

The impact of multiple pregnancies in late preterm infants has been widely evaluated. Most multiple pregnancies have the outcome of late premature birth, and are also associated with a series of fetal and maternal complications not related to prematurity, such as twin-twin transfusion syndrome, intrauterine growth restriction and the development of preeclampsia (6, 7).

The health risks for both the mother and the infant that are related to advanced maternal age are not well-documented. However, advanced maternal age contributes to an increase in cases of gestational arterial hypertension (6). Gestational arterial hypertension is a frequent cause of intrauterine growth restriction (RCIU), so premature delivery may be necessary (8). Also, this factor is associated with chromosomal abnormalities, pregnancy disorders, chronic diseases, the need for the use of assisted reproduction technology (ART), multiple births and cesarean sections, all of which may contribute to the global increase in premature births (7, 8).

Ultrasound fetal imaging to determine gestational age can be difficult in obese and overweight women. In these pregnancies, maternal obesity along with fetal macrosomia can lead to an overestimation of gestational age (7). This lack of precision can make it difficult for health professionals to make decisions, especially under adverse maternal conditions where there is a need to determine the optimal time of birth. Both fetal macrosomia and large neonate for gestational age are associated with an increased risk of traumatic birth and associated complications, which is why childbirth is brought forward in time through cesarean section or induction of birth. Maternal obesity is strongly associated with gestational diabetes, type 2 diabetes, gestational hypertension, cardiovascular disorders and many other chronic maternal diseases, all of which are risk factors for late premature births.

Premature rupture of membranes is responsible for one-third of premature births, and is another factor that contributes to the increase in late premature births (9). Choriodecidual infection is recognized as a fundamental pathophysiological mechanism of premature rupture of membranes and late premature delivery, as well as low socioeconomic status, smoking, sexually transmitted infections (STIs), multiple pregnancies, vaginal bleeding, and polyhydramnios (10). The practical guidelines published by ACOG recommend the use of tocolytics and corticosteroids for the treatment of premature labor until 34 weeks of gestation (11). When premature rupture of membranes occurs after 34 weeks, premature birth seems to be the most prudent strategy. The premature onset of labor, due to the RPM, represents a RR of three times greater and an adjusted OR of nine times greater, for the admission of neonates to intensive care units (12).

MATERIALS AND METHODS

Ethical Considerations

The respective approval of the Ethics Committee of the Hospital Universitario de the Valle and the Universidad del Valle for the realization of this study was fulfilled.

Pregnant women who agreed to participate in the study gave authorization by an informed consent document, designed following the required ethical regulations.

Design

An analytical observational case and control study conducted in Cali, Colombia, at the Hospital Universitario del Valle, which is a public institution providing level III services to mainly a low-income population, during the 2013–2014 period in Santiago de Cali. Newborns between 34 and 36 weeks of gestational age calculated by the Ballard test, selected at birth in the institution during the study period, as well as newborns that were defined as controls according to the selection criteria: born between week 37 and 40 of pregnancy in the institution. If they presented any morbidity, they entered intensive care. Exclusion criteria: Infants with major congenital malformations and mothers who would not accept to participate in the study.

To calculate the sample size, the Schlesselman formula was used for case-control studies, and taking into account the criteria of $\alpha = 0.05$ and $\beta = 0.10$. For the estimation of the sample size an occurrence of late preterm of 15% was considered. The aim was to obtain an OR of 3 and a case-control ratio 1: 1 (taking into account a loss of participants of 20% due to incomplete information). Under these considerations, a sample size of 448 infants was obtained, where 224 correspond to the case group and 224 correspond to the controls. The cases were selected by intentional sampling; newborns that met the study inclusion criteria were selected. We searched the clinical records of the database that manages the Epidemiology area of the Hospital Universitario del Valle of all births from January 2013 to December 2014. The controls were selected from the birth and cesarean section registry of the Obstetrics-Gynecology Unit. Infants from 37 to 42 weeks of gestation were identified, and each case was paired with a control with the nearest gestational age. Infants with any morbidity entered the neonatal intensive care unit for the treatment according to the institution's protocols.

The data obtained from the clinical history, both the cases and the controls, were entered into an Epi Info version three data collection program, information that was subsequently exported to the STATA 13 software, for statistical analysis.

Analysis Plan

The categorical variables generated were examined in an exploratory univariate analysis in which absolute frequencies were expressed, followed by a bivariate analysis applying the Chi-square test or Fisher's exact test, as appropriate. The strength of the association (OR) and its 95% confidence intervals (95% CI) were determined between the dependent and independent variables.

The respective adjusted OR of each group of variables was determined, once analyzed and ruling out possible interactions and confusion effects between the different factors and covariates considered. To adjust a multiple logistic regression model was implemented. To select the variables included in each of the models, the procedure *stepwise* of successive steps forward (*Forward)* was used.

TABLE 1 | Clinical and sociodemographic characteristics of puerperal women with late preterm infants and term infants.

Variable		Frequency	Percentage
Age of the Mother (years)	<19	96	22.64
	19–35	286	67.45
	>35	42	9.91
Ethnicity of the mother	Mestizo	258	60.85
	Afro	149	35.14
	Indigenous	17	4.01
Level of schooling of the mother	None	38	9.00
	Primary complete	222	52.61
	Secondary complete	130	30.81
	Technical	32	7.50
Marital status of the mother	With partner	301	70.99
	Without partner	123	29.01
Current pregnancy desired	Desired	145	34.69
	Not desired	273	65.31
Number of prenatal controls performed by the mother	Prenatal controls <3	416	98.35
	Prenatal control 4–7	5	1.18
	Control >8	2	0.47
Gestacional age of the newborn	34	46	10.85
	35	65	15.33
	36	105	24.76
	Term	208	49.06
Birth way of the newborn	Vaginal	137	32.31
	Cesarean section	287	67.69
Maternal morbidity during pregnancy	None	114	26.89
	Hypertensive disorder	242	57.08
	Obstetric	68	16.04
Newborn weight at birth	<1,500	8	1.90
	1,500–2,500	173	41.00
	>2,500	241	57.11
Newborn weight type	Small	41	9.67
	Suitable	351	82.78
	Large	32	7.55
Type of newborn morbidity	None	254	59.91
	Neurological	52	12.26
	infectious	118	27.83
Primary maternal parity	Primip1	166	39.15
	Multiparous 2–5	248	58.49
	Large multiparous ≥6	10	2.36
Gender of the newborn	Female	213	50.35
	Male	210	49.65

RESULTS

The general characteristics of the population are described in **Table 1**.

Table 1 corresponds to the univariate analysis of maternal and newborn variables in cases and controls, were we evaluated the frequency of events analyzed. It was evidenced that 22.64% of the women were teenagers and 35.14% had Afro ethnicity. Overall,

TABLE 2 | Comparative analysis between late preterm infants and term infants.

Variable	Category	Cases (212)%	Controls (212)%	*P*	OR 95% CI
Mother's age	<19 years	49(23.11)	47 (22.17)		1
	19–35	140(66.04)	146 (68.87)	0.72	0.91 (0.57–1.46)
	> 35	23(10.85)	19 (8.96)	0.68	1.16 (0.56–2.40)
Ethnicity of the mother	Mestizo	125(58.96)	133 (62.74)		1
	Afro	81 (38.21)	68 (32.08)	0.25	1.26 (0.84–1.89)
	Indigenous	6 (2.83)	11 (5.19)	0.29	0.58 (1.20–1.61)
Mother's school level	None	21 (9.91)	17 (8.10)		1
	Primary complete	113 (53.30)	109 (51.90)	0.61	0.83 (0.42–1.67)
	Secondary complete	62 (29.25)	68 (32.38)	0.41	0.73 (0.35–1.52)
	Technical	16 (7.55)	16 (7.62)	0.66	0.80 (0.31–2.07)
Marital status of the mother	With partner	146 (68.87)	155 (73.11)		1
	No partner	66 (31.13)	57 (26.89)	0.33	1.22 (0.8–1.87)
Current pregnancy desired	If wanted	65 (30.95)	80 (38.46)		1
	Did not want	145 (69.05)	128 (61.54)	0.10	1.39 (0.93–2.08)
Method of birth	Vaginal	48 (22.64)	89 (49.98)		1
	Cesarean section	164 (77.36)	123 (58.02)	0.00	2.47 (1.62–3.76)
Maternal morbidity during pregnancy	None	44 (20.75)	70 (33.02)		1
	Hypertensive disorders	124 (58.49)	118 (55.66)	0.02	1.67 (1.06–2.63)
	Obstetric events	44 (20.75)	24 (11.32)	0.00	2.91 (1.56–5.44)
Newborn weight type	Small	28 (13.21)	13 (6.13)		1
	Adequate	167 (78.77)	184 (86.79)	0.01	0.42 (0.21–0.84)
	Large	17 (8.02)	15 (7.08)	0.18	0.52 (0.20–1.36)
Newborn resuscitation maneuver	Positive pressure ventilation	130 (61.32)	190 (89.62)		1
	Oxygen at free flow	38 (17.92)	17 (8.02)	0.00	3.26 (1.76–6.03)
	Cardiac massage	42 (19.81)	5 (2.36)	0.00	12.27 (4.73–31.86)
	Orotracheal intubation	2 (0.94)	0 (0)	0.00	3.09 (1.93–4.98)
Newborn morbidity type	None	100 (47.17)	154 (72.64)	0.00	0.66 (0.51–0.84)
	Neurological	31 (14.62)	21 (9.91)		1
	Infectious	81 (38.21)	37 (17.45)	0.00	1.86 (1.47–2.34)
Primary maternal parity	Primipara 1	87 (41.04)	79 (37.26)		1
	Multiparous 2–5	116 (54.72)	132 (62.26)	0.26	0.79 (0.53-1.18)
	Great multiparous 6 or more	9 (4.25)	1 (0.47)	0.04	8.17 (1.0–65.96)

65% did not want a pregnancy and 98% had inadequate prenatal control. Furthermore, hypertensive disorders represented the highest proportion of maternal morbidity and infections in the newborn.

Bivariate Analysis

A bivariate analysis using the Chi square test or the Fisher exact test, as appropriate. The strength of the association (OR) and its 95% confidence intervals (95% CI), between the dependent variable and the independent ones, were determined. With this methodology, different associations were identified for each group of variables (**Table 2**).

Table 2 corresponds to bivariate analysis of cases and control, identifying significant results associated with late prematurity. Late preterm newborns required a greater chance of resuscitation (Oxygen 3.26 times, cardiac massage 12.27 times and orotracheal intubation 3.09 times). The mothers showed a higher probability of 2.91 times of obstetric pathology (such as pelvic disproportion, multiple pregnancy and the threat of preterm birth), and births by cesarean section by 2.47 times. Moreover, there was a greater maternal morbidity due to hypertensive events by 1.67 times in mothers of late preterm newborns.

Final Model

In the selection process of the predictor variables, the results of the bivariate analysis were taken into account, using the variables that indicated a significant association (0.02 alpha) with the event, to subsequently apply the logistic model with the selection technique of forward variables (*Forward*).

Table 3 shows the results posterior to the adjustment in a multiple logistic regression model. There is a statistically significant association of late prematurity with the presence of maternal morbidity during pregnancy (specifically when presenting with hypertensive disorders and obstetric alterations) (ORa 1.37 95%IC 1.14–1.65) and birth via cesarean section (ORa 4.17 95% CI 2.50–6.98). Also, late preterm infants have a

TABLE 3 | Results posterior to the adjustment in a multiple logistic regression model.

Variable	Adjusted OR	*P*	(95% CI)
Newborn resuscitation maneuver	3.23	0.00	2.09–4.99
Newborn Birth route	4.17	0.00	(2.50–6.98)
Maternal morbidity during pregnancy	1.37	0.00	(1.14–1.65)
Type of newborn morbidity born	1.26	0.07	(0.97–1.64)

greater chance of requiring resuscitation maneuvers (ORa 3.23 95% CI 2.09–4.99) and have a close to statistical significance a greater opportunity to present neonatal morbidity (ORa 1.26 95% CI 0.97–1.64).

DISCUSSION

Reports of premature births in the world are close to 12%, of which 71% correspond to late premature babies, 12.6% moderate premature babies, 10.1% early premature babies, and 5.9% extreme premature (13, 14). In Colombia, the prematurity rate represents a 20%, much higher than reported in other high-income countries (15). Dimitriou et al. in Greece (16) investigated morbidity determinants in 548 late preterm infants, through a prospective cohort study, finding that 165 of the infants had morbidity during hospitalization associated with childbirth. The most frequent morbid conditions were respiratory distress (25.4%) and hyperbilirubinemia that required phototherapy (25.2%). Early gestational age (OR 7.32 for 34 weeks and 3.05 for 35 weeks gestation) and Small PEG for gestational age (OR 2.69), were recognized as factors associated with a significant impact on neonatal morbidity. In Turkey, Özlem et al. (17) (2005–2007) developed a study that included 252 late preterm infants, presenting a mortality rate of 2.3%, whilst no term neonates died in the same period. When compared to term infants, late preterm infants were 11 times more likely to develop respiratory failure, 14 times more likely to have feeding problems, 11 times more likely to have hypoglycemia, three times more susceptible of being readmitted and 2.5 times more likely to require re-hospitalization.

Likewise, in a prospective multicenter control case study in Uruguay, Moraes et al. (18) described that respiratory distress syndrome had a statistically significant difference in late preterm infants with OR 4.57 (1.95–10.82) but not for those of 37 weeks. There were 19 cases of respiratory distress (26.3%) of which 17 (23.6%) corresponded to transient tachypnea, a case of hyaline membrane disease and a case of severe pulmonary hypertension (1.38%). Also, more metabolic disorders, hypoglycemia, hypothermia and jaundice were observed with statistical significance. Moreover, in Chile, Schonhaut et al. (19), studied neonatal morbidity in late preterm infants, which corresponded to 5.12% of live births of the year 2009. Premature infants have an increased risk of presenting respiratory distress syndrome (RR 17.3), apnea of prematurity (15.7) and third hypothermia (RR 10.8). In the present study, the probability of late prematurity was associated 3.23 times to higher requirement of resuscitation and 1.26 times (near the statistical significance) of developing early neonatal morbidity. Thus, as mentioned by previous publications and by this current investigation, late premature babies show a higher probability of requiring reanimation, with an ORa 3.23 (95% CI 2.09–4.99) and have a greater opportunity (close to statistical significance) of presenting neonatal morbidity (ORa 1.26 95% CI 0.97–1.64).

Furthermore, various investigations associate (20, 21) the development of morbidities during pregnancy with the probability of having a preterm birth. These associated morbidities were gestational diabetes and maternal hypertensive disease. The present study analyzed the factors significantly associated with late prematurity, such as morbidities of the mother (hypertensive disorders) and obstetric complications (disproportion pelvic head, multiple pregnancies, and the threat of preterm birth), that were correlated with an increased opportunity of 1.37 times of premature birth (95% CI 1.14–1.65). It is important to note that these factors can be recognized and identified early in the pregnancy, which indicates a way to prevent late prematurity.

A study with an ecological design (22) carried out in developed countries showed that places of high rates of late premature and premature births are associated with low rates of stillbirths and neonatal mortality. The authors consider that when cesarean birth is required due to medical indications in the preterm, they are generally beneficial since they had a significant risk of mortality. In this study, late premature births were also significantly associated by 4.17 times (95% CI 2.50–6.98) to birth by cesarean section, which was indicated for medical reasons, as many patients are referred to this institution of greater complexity due to their serious condition.

Some studies (23, 24) significantly associate late prematurity and increased neonatal morbidity to social factors such as low income or low schooling. In the present study, no association was observed to income or schooling, since the population that comes to this hospital as a whole belongs to low-income groups. Concerning their schooling, only 52% reached primary school, 31% achieved up to secondary school and 9% have no studies (in both groups). Furthermore, inadequate prenatal control (<3 prenatal controls) has also been described (21) as a factor associated with late prematurity. In the present study no association was evidenced, as 98% of the population treated in this institution in both groups did not receive adequate prenatal control.

Late premature babies were historically perceived with similar morbidity as full-term babies. However, several studies have shown that late premature babies manifest an increase in early complications much more frequently than full-term children. In this investigation, newborn morbidity was 1.26 times greater when compared to full-term children (ORa 1.26 95% CI 0.97–1.64), this is not significant due to sample size. However, it is pertinent to mention it, as other articles have investigated its importance in late prematurity. In Jobe (25) "A new disease: late preterm infants" and Davidoff MJ's publications (1), there is a growing concern for this group of infants, which is driven by the

experience of health personnel directly involved, an increasing number of cases from 1990 to 2005, poor results related to health interventions in the first hospitalizations and an increase in medical care during its first year.

On account of the studies mentioned and the results observed in this research, the population of late premature infants has a greater association of short and long term complications compared to those born at term. Late premature infants have significant morbidity (its outcome non-lethal in the majority of investigations), are associated with disabilities, decreased quality of life and increased costs to the health services (25, 26).

A limitation of the study was using the medical records as a source, as some of these were incomplete, which did not allow for sufficient information in many of the variables analyzed. This significantly effects the result. Another limitation is that more than 90% of the population studied had low income, which did not allow social comparisons, as other studies have done.

CONCLUSIONS

Birth of late premature infants has an important financial impact, derived from antepartum management, childbirth, neonatal treatment, and requirements of special medical care during their follow-up for several months. This forces an implementation of public health actions in the prevention of late preterm birth, such as improving access to adequate prenatal control. In this investigation, a very low percentage of prenatal control is observed and this consequently favors maternal morbidity. When maternal morbidity is not detected, it increases the chance of late preterm births with complications that require hospitalization, raises the probability of long-term effects and elevates financial costs for the health system.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Comité de ética de la Universidad del Valle; Comité de ética del Hospital Universitario del Valle. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

JT-M: study design and analysis and writing of the manuscript. CJ-F: study design and writing of the manuscript. RO: data collection and analysis. DC and DM: data collection. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Davidoff MJ, Dias T, Damus K, Russell R, Bettegowda VR, Dolan S, et al. Changes in the gestational age distribution among US singleton births: impact on rates of late preterm birth, 1992 to 2002. *Semin Perinatol.* (2006) 30:8–15. doi: 10.1053/j.semperi.2006.01.009
2. Raju TNK, Higgins RD, Stark AR, Leveno KJ. Optimizing care and outcome for late preterm (near term) infants: a summary of the workshop sponsored by the National Institute of Child Health and Human Development. *Pediatrics.* (2006) 118:1207–14 doi: 10.1542/peds.2006-0018
3. Engle W. Infants born late preterm: definition, physiologic and metabolic immaturity, and outcomes. *NeoReviews.* (2009)10:e280–6. doi: 10.1542/neo.10-6-e280
4. Benjamin Morse S, Zheng H, Tang Y, Roth J. Early school-age outcomes of late preterm infants. *Pediatrics*. (2009) 123:e622–9. doi: 10.1542/peds.2008-1405
5. Engle WA, Tomashek KM, Wallman C. "Late-preterm" infants: a population at risk. *Pediatrics.* (2007) 120:1390–401. doi: 10.1542/peds.2007-2952
6. Dobak WJ, Gardner MO. Late preterm gestation: physiology of labor and implications for delivery. *Clin Perinatol.* (2006) 33:765–76. doi: 10.1016/j.clp.2006.09.001
7. Raju T. Epidemiology of late preterm (near-term births) births. *Clin Perinatol.* (2006) 33:751–63. doi: 10.1016/j.clp.2006.09.009
8. Adeniyi-Jones S. Intrauterine growth retardation. In: Spitzer AR, editor. *Intensive Care of the Fetus and Neonate*. Philadelphia: WB Saunders (1996). p. 137.
9. Escobar GJ, Greene JD, Hulac P, Kincannon E, Bischoff K, Gardnermn, et al. rehospitalization after birth hospitalization: patterns among infants of all gestations. *Arch Dis Child.* (2005) 90:125–31 doi: 10.1136/adc.2003.039974
10. Mercer BM. Preterm premature rupture of membranes. *Obstet Gynecol.* (2003) 190:1313–21. doi: 10.1016/s0029-7844(02)02366-9
11. American College of Obstetricians and Gynecologists, Society for Maternal-Fetal Medicine. Obstetric care consensus No. 6: Periviable Birth. *Obstet Gynecol.* (2017) 130:e187–99. doi: 10.1097/AOG.0000000000002352
12. Carter M, Xenakis E, Holden A, Dudley D. Neonatal intensive care unit admissions and their associations with late preterm birth and maternal risk factors in a population-based study. *J Maternal Fetal Neonatal Med.* (2012) 25:343–5. doi: 10.3109/14767058.2011.576723
13. Ramachandrappa A, Jain L. Chapter 42: the late preterm infant. In: Martin RJ, Fanaroff AA, Walsh M, editors. *Fanaroff & Martin's Neonatal-Perinatal Medicine, Diseases of the Fetus and Infant.* Philadelphia, PA: Elsevier (2015).
14. Chawanpaiboon S, Vogel JP, Moller AB, Lumbiganon P, Petzold M, Hogan D, et al. Global, regional, and national estimates of levels of preterm birth in 2014: a systematic review and modeling analysis. *Lancet Glob Health.* (2018) 7:37–46. doi: 10.1016/S2214-109X(18)30451-0
15. Available online at: https://www.dane.gov.co/index.php/calendario/icalrepeat.detail/2019/12/20/3944/-/estadisticas-vitales-nacimientos-y-defuncionesColombia
16. Dimitriou G, Fouzas S, Georgakis V, Vervenioti A, Vassilis G. Papadopoulos, george decavalas, stefanosmantagos. Determinants of morbidity in late preterm infants. *Early Hum Dev.* (2010) 86:587–91 doi: 10.1016/j.earlhumdev.2010.07.011

17. Kalyoncu O, Aygü NC, Erhan C, Etinoglu, Sükrücüködü K. Neonatal morbidity and mortality of late-preterm babies. *J Mater Fetal Neonatal Med.* (2010) 23:607–61 doi: 10.3109/14767050903229622
18. Mario M, Mónica P, Nolberto M. Morbilidad en pretérminos tardíos: estudio prospectivo caso control multicéntrico. *Arch Pediatr Urug.* (2009) 80:196–202. Available online at: http://www.scielo.edu.uy/scielo.php?script=sci_arttext&pid=S1688-12492009000300005&lng=es
19. Schonhaut L, Pérez M, Astudillo J. Prematuros tardíos: un grupo de riesgo de morbilidad a corto y largo plazo. *Rev Chil Pediatr.* (2012) 83:217–23. doi: 10.4067/S0370-41062012000300002
20. Araújo BF, Zatti H, Madi JM, Coelho MB, Olmi FB, Canabarro CT., et al. Analysis of neonatal morbidity and mortality in latepreterm newborn infants. *J Pediatr (Rio J).* (2012) 88:259–66.196 doi: 10.2223/JPED.2196
21. Brown HK, Speechley KN, Macnab J, Natale R, Campbell MK. Biological determinants of spontaneous late preterm and early term birth: a retrospective cohort study. *BJOG.* (2015) 122:491–9. doi: 10.1111/1471-0528.13191
22. Lisonkova S, Sabr Y, Butler B, Joseph KS. International comparisons of preterm birth: higher rates of late preterm birth are associated with lower rates of stillbirth and neonatal death. *BJOG.* (2012) 119:1630–9. doi: 10.1111/j.1471-0528.2012.03403.x
23. Ruth CA, Roos N, Hildes-Ripstein E, Brownell M. The influence of gestational age and socioeconomic status on neonatal outcomes in late preterm and early term gestation: a population based study. *BMC Preg Childbirth.* (2012) 12:62. doi: 10.1186/1471-2393-12-62
24. Abdel Razeq NM, Khader YS, Batieha AM. The incidence, Risk factors, and mortality of preterm neonates: a prospective study from Jordan. *Turk J Obstet Gynecol.* (2017) 14:28–36. doi: 10.4274/tjod.62582
25. Laughon SK, Reddy UM, Sun L, Zhang J. Precursors for late preterm birth in singleton gestations. *Obstet Gynecol.* (2010) 116, 1047–55. doi: 10.1097/AOG.0b013e3181f73f97
26. Vanin LK, Helen Z, Thaise S, Nunes RD, de Siqueira LBS. Maternal and fetal risk factors associated with late preterm infants. *Rev Paul pediatr.* (2020) 38:e2018136. doi: 10.1590/1984-0462/2020/38/2018136

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Associations of History of Displacement, Food Insecurity, and Stress With Maternal-Fetal Health in a Conflict Zone: A Case Study

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Background: In populations with a history of conflict, early identification of pregnant women who are at risk of adverse pregnancy outcomes is challenging, especially if sonography is not available. We evaluated the performance of symphysis-fundal height (SFH) for identification of high-risk pregnancies and investigated if food security and diet quality, clinical biomarkers, and stress were associated with SFH and two known indicators of maternal-fetal well-being, sonography-estimated fetal weight and amniotic fluid index (AFI).

Methods: For this cross-sectional study, 61 women with high-risk pregnancies were recruited after referral to the obstetrics and gynecology unit at San José Hospital in Popayán, Colombia. Multiple stepwise linear and ordered logistic regressions were used to identify associations of SFH, sonography-estimated fetal weight and AFI classification with history of displacement, food insecurity, post-traumatic stress symptoms as well as biopsychosocial risk evaluated through the Colombian risk scale.

Results: History of displacement was associated with lower SFH Z-scores, but higher hemoglobin, taking iron supplements and a higher diastolic blood pressure were associated with higher SFH Z-scores. SFH was also associated with AFI but not with sonography-estimated fetal weight. Stress indicators were associated with a higher AFI. In contrast family support, an element of the Colombian biopsychosocial risk assessment, was associated with a higher sonography-estimated fetal weight, whereas more hours of sleep/day were associated with lower sonography-estimated fetal weight.

Conclusion: SFH was not only associated with biological factors known to affect maternal/fetal health but also with history of displacement, thus validating its use in conflict areas for pregnancy assessment. Associations of biopsychosocial stressors with maternal-fetal outcomes highlight the need for a systematic assessment of stress in pregnant women from conflict zones.

Keywords: symphysis-fundal height, sonography-estimated fetal weight, amniotic-fluid index, internal displacement, stress scale

INTRODUCTION

The World Bank reports that ∼2 billion people reside in conflict-affected areas (1). Presently, the United Nations recommends a focus on maternal and neonatal health in conflict zones if targets of the Sustainable Development Goals are to be achieved (2). Colombia has been experiencing armed conflict for over three decades (3) with widespread internal displacement (4) of 6 million people because of violence (5). Internal displacement due to conflict poses physical and mental health risks and leads to disruption of protective factors like community and family networks (1). There is also evidence of slower progress in achieving optimal levels of antenatal care in conflict zones (6).

The impact of conflict on pregnancy outcomes is difficult to measure. Obstetric ultrasound is considered the most reliable and objective way to monitor fetal growth and assess gestational age (GA) (7) and has been successfully used in the prediction of fetal distress in developing countries by measuring fetal movements and amniotic fluid index (8). However, its accessibility, feasibility, and the disclosure of the infant sex and health limits its use in some populations (9). In settings where obstetric ultrasound is not available, a common practice is to measure symphysis-fundal height (SFH) (10). Currently, WHO advocates for further research to determine the association of SFH with fetal growth and risk factors for perinatal morbidity particularly in settings where antenatal ultrasound is not available (11). In Latin America, a reference SFH chart, developed by the Latin American Centre of Perinatology and Human Development (CLAP) is the commonly used (12). Recently, new standards for SFH were adopted by the Intergrowth-21 project, a multicenter and multiethnic study of 8 geographically different countries (13) but studies on the application of these new SFH standards in Latin America are lacking.

Since biopsychosocial factors can contribute to adverse pregnancy outcomes (14), the Colombian Ministry of Health has adopted a biopsychosocial score used to screen pregnant women on the basis of their medical conditions, degree of stress and social/family support, in order to better identify those women at high risk of adverse pregnancy outcomes (15). However, the prevalence of pregnancy complications, particularly small-for-gestational-age babies, continues to be higher in Colombian conflict zones compared with the rest of the country (16), given exposure to conflict-related stress and food insecurity beyond the general difficulties of mothers in low-medium income countries.

Therefore, the objective of this study was to explore if food security and diet quality, clinical biomarkers, history of displacement, and markers of stress were associated with three indicators of maternal-fetal well-being: (1) symphysis-fundal height (SFH), (2) sonography-estimated fetal weight, and (3) sonography-calculated amniotic fluid index (AFI). Stress was measured using scores from both the Colombian biopsychosocial risk scale (15) and the adapted conflict-related stress score (17), in order to assess the possibility that our population may be experiencing post-traumatic stress. We also explored the possibility that SFH could be a useful biomarker to detect pregnancies at risk of adverse outcomes in settings with no access to sonography.

METHODS

Ethics and Study Population

Ethics approval was obtained from the Scientific Research Ethics of San José Hospital in Popayán, Colombia, by endorsement certificate No. 02 dated April 9, 2018. Participants gave written informed consent in accordance with the Declaration of Helsinki, the Belmont Code and the Federal Regulations Code from the US National Institutes of Health, as stated in Colombian Ministry of Health's Act 008430.

Through a purposive sampling process, we aimed to obtain a representative sample of women from urban and rural areas, from different municipalities and with different risk factors for adverse pregnancy outcomes. The research team estimated that such a sample could be achieved by recruiting women identified as having a high-risk pregnancy during consultation over a 1-month time period at the obstetrics and gynecology service which runs 3 times/week at the San José Hospital in Popayán, the main referral center for patients in the subsidized health care system. Pregnant women of any GA with singleton pregnancies attending the out-patient high-risk-pregnancy clinic between April and May 2018 were asked to participate. Mothers were approached by the principal investigator (DGF) while they were waiting for their appointment. Mothers were informed about the aim of the study, the time that the interview would occur, the volunteer nature of their participation and the non-interference with their regular pregnancy follow-up and treatment. They were told that no financial compensation was provided but that, after understanding their dietary habits, individualized nutritional counseling would be provided.

We were able to recruit 61 mothers referred to this specialized follow-up clinic because of a high-risk pregnancy due to one or more of the following: adolescent pregnancy, multiparity, history of preterm delivery, and morbidities such as hypertensive disorders of pregnancy, anemia, gestational diabetes, or auto-immune disorders (hypothyroidism). All participants were interviewed by the principal investigator after their ambulatory clinical evaluation and none had a critical condition. Of the 61 women, 57 were beyond the 16th week of pregnancy and 48 were >22nd week of pregnancy, the minimal GAs for comparison with the INTERGROWTH standards for SFH and fetal weight growth, respectively (13). We were able to obtain delivery information from 57 women and had missing data on hemoglobin ($n = 2$) and hematocrit ($n = 2$). Multiple linear regression models included the STATA complete-case analysis function (18) which allowed us to both maximize the sample size of each final model and confirm the randomness of missing data using Little's chi-squared test (19).

Maternal Health Evaluation

Information on obstetric history including risk factor antecedents, history of urogenital infections in the current pregnancy, hemoglobin and hematocrit, weight and height measurements and sonography information were obtained from clinical charts on the day of the interview by the principal investigator. Maternal weight-for-height was compared with the

Pan-American Health Organization standards for GA to classify women as underweight, normal, or overweight (12).

Treating physicians took systolic (SBP) and diastolic (DBP) blood pressure using calibrated mercury sphygmomanometers and arm-cuffs adjusted to maternal arm circumference, with the mother in sitting position. The average of blood pressure taken in both arms in extended position at the level of the heart was recorded following recommendations of the Colombian Ministry of Health guidelines (20). Using SBP and DBP from clinical files, mean arterial pressure (MAP), a known risk factor for hypertensive disorders of pregnancy, was calculated as DBP + 1/3 (SBP—DBP) (21) and cut-offs from the United Nations' Women's Health and Education Center were used to define elevated MAP (22). Pulse pressure (PP) was calculated as the difference between SBP and DBP and was considered elevated if >68 mmHg and low if <42 mmHg (23). Anemia was defined as hemoglobin <11 g/dl (24) and normal hematocrit was defined as 35–44% during the first, 30–39% during the second, and 28–40% during the third trimester (25).

Maternal-Fetal Examination

Mothers at the high-risk pregnancy unit had been referred from peripheral health centers, where basic laboratory and sonography studies were performed. Gestational age at consultation was determined using these early ultrasounds when available. If early ultrasound was not available, date of the last menstrual period was used to estimate GA. In case of inconsistency between date of last menstrual period with a later ultrasound or unreliable last menstrual period (e.g., irregular menstrual cycles, breastfeeding, or using hormonal contraception), the most recent sonography was used.

SFH was measured by physicians at the obstetrics-gynecology department, with the patient lying flat on a bed with her legs extended, using a flexible, non-elastic standard measuring tape extended from the middle of the upper border of symphysis pubis to the highest point of the uterine fundus. SFH in centimeters measured on the day of interview was recorded from hospital records and later translated into Z-scores and centiles using international INTERGROWTH standards (13).

At the time of the interview, most women had obstetric ultrasound performed by a trained obstetrician following the Colombian Ministry of Health Guidelines (20). Sonography-estimated fetal weight and AFI were obtained from hospital records. Sonography-estimated fetal weights (grams) were translated into Z-scores and centiles using international INTERGROWTH standards (26). AFI values <5 cm were considered as oligohydramnios (27) and those >24 cm were considered as polyhydramnios (28). As AFI varies according to GA, we classified AFI as falling into centiles (2.5, 10th, 50th, or 97.5) for GA as described by Machado et al. (29). We also categorized AFI into its <25th, 25th−75th, and ≥75th centiles. A later review of clinical files in December 2019 allowed us to record GA at delivery and birth weights. If mothers did not deliver at the San José Hospital, delivery information was taken from Cauca's Secretary of Health Database. Information on Apgar scores at 1 and 5 min was recorded. Preterm births (<37 weeks GA) and low birth weights (<2,500 g) (30) were identified.

Socio-Demographic and Psychosocial Questionnaires

Participants completed the following socio-demographic and diet-related questionnaires: (1) a socio-demographic and household assessment (31), (2) the Colombian household food security scale (32), and (3) a food frequency questionnaire based on national guidelines from the Colombian Institute of Family Welfare (Instituto Colombiano de Bienestar Familiar ICBF) (33).

The Colombian biopsychosocial risk scale, which is part of the national guidelines for pregnancy assessment follow-up, was obtained from hospital records. Among all biologic risk factors assessed by the scale, the following were present in our participants: age <16 years (1 point), >35 years (2 points), first pregnancy (1 point), multiparity (>5 gestations, 2 points), previous cesarean section (1 point) or pelvic surgery (1 point), history (1 point), or current (2 points) gestational hypertension, history (1 point) or current (2 points) gestational diabetes, as well as the presence of autoimmune disease (3 points) or presence of anemia (1 point). Psychosocial risks included the presence of emotional distress, depressive mood, and anxiety symptoms (2 or more "intense" items = 1 point). For family support, mothers were asked if they were satisfied with their family or partner support, and answers were recorded in a scale of 0–3 (15). Values 0–1 were considered as low family support. A total scale ≥3 across all questions was classified as high biopsychosocial risk.

In order to determine whether women had experienced a "history of displacement" in response to conflict over the past 50 years, mothers were asked if they had "ever been in a situation of forced displacement due to the armed conflict."

Women also answered a stress questionnaire adapted from a previously validated Afghan Symptom Checklist score of locally-relevant symptoms of post-traumatic stress in a conflict zone (17). With the adapted score, we assessed the intensity (0 = none, 1 = occasionally, 2 = sometimes, 3 = often) of seven items: recall of stressful events; dreaming about stressful events or nightmares; presence of symptoms like sweating, shaking, or tachycardia (anxiety-like symptoms) when recalling or affected by stressful situations; lack of interest in daily activities; not being able to feel love toward their loved ones; easily startled; and difficulty sleeping. The average score was used to classify women as having no stress (score = 0), mild (score >0 and ≤1), moderate (score >1 and ≤2) and severe stress (score >2). The duration of sleeping at night was also recorded using a scale of 0 (<3 h), 1 (≥3 and <6h), 2 (≥6 and ≤8 h), and 3 (>8 h).

Statistical Analyses

All statistical analyses were performed using STATA16 (StataCorp, TX, USA), including correlations of food insecurity with history of displacement and with elements of the adapted conflict-related stress questionnaire, the Colombian biopsychosocial risk score, and with weekly intake of foods.

To evaluate the usefulness of SFH as an indicator of fetal growth, we used two approaches: (1) Spearman's correlations between SFH Z-scores and Z-scores of both sonography-estimated fetal weight and birth weights and (2) a multiple regression model for SFH (cm) with sonography-estimated fetal

weight (kg) and AFI (cm) as independent variables, while controlling for maternal weight-for-height classification.

In order to determine if maternal characteristics and environmental factors, supplementation/medication variables, dietary variables, and biological factors (presence of infections, hemoglobin, and hematocrit concentrations) were associated with SFH, sonography estimated fetal weight or AFI, we used a three step process. First, for each binary variable (yes/no) where the condition had ≥10 "yes" observations, mean Z-scores of SFH, and sonography-estimated fetal weight were compared using Student's *T*-tests. Second, we assessed associations of our independent variables with SFH Z-scores and sonography-estimated fetal weight Z-scores using linear regressions and with AFI (<25th, 25–75th, and >75th) using ordered logistic regressions. Third, of the six clusters of independent variables, those with a $P \leq 0.10$ that were not collinear were tested in a backwards stepwise process for inclusion in a single "core" multiple regression model. Then, using the sixth cluster, each element of the stress score, each element of the biopsychosocial score, the composite stress score, and high biopsychosocial risk were entered separately because of collinearity among these variables. The significance level for removal from the model ($P < 0.10$) allowed us to obtain final models with a maximum of 5 variables. If a continuous dependent variable had non-linearity issues in final models, the variable was transformed into its ordinal equivalent as 1: <25th centile, 2: ≥25th and ≤75th centile, and 3: >75th centile. Final models with significant stress elements are reported. Missing data were not imputed and complete case analyses resulted in models with $n = 49$–52. Similar percentile- and bias-corrected confidence intervals of bootstrapping reproduced models were used to confirm the stability and unbiased selection of variables in our linear regression models (34). STATA's margins command was used to estimate marginal effects in our ordered logistic regression models (35). Final regressions were evaluated for collinearity (variance inflation factor <10), stability of coefficients (condition number <30), and linearity of associations (augmented component-plus-residual plots and weighted regression lines).

RESULTS

Social and Household Characteristics

Maternal characteristics are summarized in **Table 1**. Mothers' age was 27 ± 7 (mean ± SD) years, had studied 11 ± 3 years. Among our participants 36.1% came from Popayán, the remainder came from 15 municipalities of Cauca and 78.7% lived in urban areas. Most women had finished high school (24.6%) or had technical or university education (44.3%). In general, all urban women benefitted from treated water (80.3%) and sanitation (88.5%), but women from more remote rural areas obtained water from wells or rural aqueducts. Most women had a stable partner (70.5%), 16.4% were not in stable unions, and 13.1% were single. Most households (72.0%) were food secure, but diet quality according to recommendations was inadequate (**Table S1**).

Clinical Assessment

Maternal and fetal health indicators are summarized in **Table 2**. Mothers had no evidence of high SBP, DBP, or PP. However, MAP was elevated in 11.5%. In contrast, a PP < 42 mmHg occurred in 67.2% of mothers. Anemia was not a problem as only 4.9% had hemoglobin values <11 g/dL and none had hemoglobin <10 g/dL. However, hematocrit exceeded normal values for GA in 42.6% of the women. Women were prescribed iron (65.6%), calcium (67.2%), and folic acid (59.0%) supplements, and 41.0% purchased multivitamin-mineral supplements.

Pregnancy and Offspring Characteristics

At interview, 3.3% women were in their first trimester, 18.0% were in their second, and most (78.7%) were in their third trimester. INTERGROWTH standards for SFH and sonography-estimated fetal weight revealed that SFH values were <10th centile in 64.1%, but only 5.9% of sonography-estimated fetal weights were <10th centile (**Table 2**, **Figures 1**, **2**). A low AFI was found in 14.0% and a high AFI in 4.0% (**Table 1**).

Assessment of Maternal Stress

None of the women reported recent displacement, but 29.5% of the mothers reported a history of displacement. However, in the adapted conflict-related stress questionnaire (**Table 3A**), 8.2% reported no stress, 72.1% had mild stress, 13.1% had moderate stress, and 6.6% reported severe stress in the current pregnancy. Of note, symptoms of both anxiety and depression and also sleep disturbances were common (**Table 3A**).

Regarding the Colombian biopsychosocial assessment tool (**Table 3B**), 65.6% had at least one of the biological risk factors that included adolescent pregnancy, grand multiparity, history of preterm delivery, gestational diabetes, and/or hypertensive disorders of pregnancy, 62.3% reported having psychological risk factors and 9.8% had low or no family support; most women (90.1%) reported moderate or high family support. However, when assessing the overall biopsychosocial risk, 24.6% were at high risk for adverse pregnancy outcomes (score ≥ 3).

Correlations of History of Displacement and Food Insecurity With Diet and Stress Variables

Importantly, history of displacement was not correlated with elements of the adapted stress questionnaire, the Colombian biopsychosocial risk score or frequency of intake of food groups. On the other hand, food insecurity score was correlated with our stress questionnaires. For the adapted stress questionnaire, higher food insecurity correlated with having no interest in daily activities, not feeling love toward relatives and friends, having difficulty sleeping, recalling stressful events, anxiety-like symptoms, being easily startled, and having difficulty sleeping. For the Colombian biopsychosocial risk scale, higher food insecurity correlated with emotional tension, with depressive mood, with neurovegetative symptoms, and with less family support. With regards to diet quality and compliance with Colombian dietary recommendations, food insecurity was correlated with lower intake of dairy products, nuts/avocados,

TABLE 1 | Socio-demographic and diet characteristics.

Place of provenance	**%**	**Food insecurity score**	**%**
Departmental capital	59.0	No food insecurity	72.1
Municipalities with mixed ethnicities[a]	19.7	Mild food insecurity	9.8
Indigenous municipalities[b]	16.4	Moderate food insecurity	8.2
Black municipalities[c]	4.9	Severe food insecurity	9.8
Household		**Food insecurity responses**	**%**
Rural	21.3	Not having sufficient money to buy food	28
Urban	78.7	An adult eats less due to lack of resources	16
Access to potable water	80.3	Decrease of meals due to lack of resources	7
Education level		Adult skips meals	5
Primary	11.5	Adult complains of hunger	8
Incomplete secondary	19.7	Adult went to bed hungry	5
Completed secondary	24.6	Bought less food for kids due to lack of resources	8
Technical/university	44.3	Child complains of hunger	3
Occupation		Child went to bed hungry	2
House	45.9	**Following diet recommendations**	
Technical/professional	19.7	Eggs everyday	67.2
Student	16.4	Dairy products everyday	45.9
Retailing	9.8	Legumes at least 2 times/week	78.7
Other	8.2	Nuts/avocados at least once/week	42.6
Marital status		Viscera once/week	32.8
Single	13.1	Fruits and vegetables with every meal	22.5
Unstable partnership	16.4	Limited processed/can meats	80.3
Stable partnership/cohabitation	70.5	Not eating junk food	75.4
Monthly income[d]		Use of vegetable oil for cooking	91.8
<1 minimum salary/month	27.8	**Number of food groups for which compliance was achieved**	
1 minimum salary/month	32.9	1–3	18.0
>1 minimum salary/month	39.3	4–6	55.7
		7–9	26.3

[a] *Mixed ethnic background refers to mestizo, indigenous and black races.*
[a] *Argelia, Bolívar, El Tambo, Mercaderes, San Sebastián, Timbío.*
[b] *Cajibío, Coconuco, Inzá, Jambaló, Morales, Piendamó.*
[c] *Patía, Guapi, Santander de Quilichao.*
[d] *In 2018, a minimum salary was 781,242 COP, equivalent to about 220 USD/month.*

legumes, fruits, and vegetables (**Table S2**), and with less hours of sleep.

Factors Associated With Fetal Growth

Comparison among means of SFH Z-scores by the presence/absence of several maternal characteristics is described in **Table 4**. Lower SFH Z scores were found in women with a history of displacement and in those taking multivitamins whereas higher SFH Z-scores occurred in women receiving iron or calcium supplements (**Table 4A**). A similar comparison for sonography-estimated fetal weight showed that Z-scores were significantly lower in women living in rural areas, those with low incomes and those who slept >8 h/day but higher in women who slept <6 h (**Table 4B**).

Symphysis-Fundal Height

SFH Z-scores were significantly correlated with sonography-estimated fetal weight Z-scores ($r_s = 0.34$, $P = 0.017$), AFI ($r_s = 0.35$, $P = 0.015$), and birth weight Z-scores ($r_s = 0.32$, $P = 0.021$), but with low correlation coefficients. However, our multiple linear regression revealed that SFH in cm was positively associated with GA (standardized β-coefficient = 0.89, $P < 0.0001$) and AFI (cm) ($\beta = 0.13$, $P = 0.023$) but not with sonography-estimated fetal weight (kg) ($\beta = 0.02$, $P = 0.72$) when adjusting for maternal weight-for-height classification (full model $R^2 = 0.85$ and $P < 0.0001$).

Determinants of Symphysis-Fundal Height Z-Scores

The stepwise model for SFH Z-scores captured 26% of the variability and showed that history of displacement was associated with lower SFH Z-score whereas higher MAP was associated with higher SFH Z-scores (**Table 5A**). In order to observe which component of MAP was associated with SFH, SBP and DBP were included in separate models because of collinearity. Higher DBP, higher hematocrit and taking iron supplements were positively associated with SFH Z-scores. This model captured 38% of variability in SFH Z-score (**Table 5B**).

TABLE 2 | Maternal and fetal health indicators.

Maternal clinical information		**Sonography measurements (*n* = 61)**	
Gestational age at interview, median (min-max)	31.4 (16.3–38.6)	Gestational age at sonography (weeks), median (min-max)	31.2 (7.4–38.2)
Weight-for-height classification (*n* = 61)		**Fetal weight (grams) in women with GA ≥20 weeks (*n* = 51),** mean ± SD	1926.4 ± 790.9
Underweight, %	9.8	Fetal weight Z-score, mean ± SD	0.07 ± 0.81
Normal weight, %	42.6	Fetal weight centiles median (min-max)	54.5 (5.4–96.9)
Overweight	47.5	Fetal weight <10th centile, %	5.9
Blood pressure measurements (mmHg) (*n* = 61)		**Amniotic fluid index (*n* = 50)**, mean ± SD	13.9 ± 4.1
Systolic blood pressure, mean ± SD	112 ± 12	Centile classification for GA	
Diastolic blood pressure, mean ± SD	68 ± 9	Amniotic fluid index <10th centile, %	14.0
Mean arterial pressure, mean ± SD	83 ± 10	Amniotic fluid index >90th centile, %	4.0
Pulse pressure, mean ± SD	43 ± 8	Low and high AFI in our population	
Elevated mean arterial pressure, %	37.7	Amniotic fluid index <25th centile, %	22.0
Pulse pressure <42 mmHg, %	57.4	Amniotic fluid index >75th centile, %	24.0
Hematological variables (*n* = 59)		**Newborn characteristics (*n* = 57)**	
Hemoglobin, g/dL, mean ± SD	12.8 ±1.2	Gestational age at delivery (weeks), median (min-max)	38.5 (29.3–41.0)
Hematocrit, percentage, mean ± SD	38.7 ± 3.8	Preterm delivery (<37 weeks), %	14.0
High Hematocrit, %	42.6	**Birth weight (g)**, mean ± SD	3052.0 ± 492.5
Anemia (hemoglobin<11 g/dL), %	4.9	Birth weight Z-score, mean ± SD	0.04 ± 0.83
Symphysis-fundal height (SFH, cm) (*n* = 53), mean ± SD	27.0 ± 5.1	Birth weight <2,500 g, %	12.3
SFH Z-score, mean ± SD	−1.53 ± 1.16	Apgar score at 1 min, ≥8, %	96.5
SFH centile, median (min-max)	7 (0–85.4)	Apgar score at 5 min, ≥8, %	100.0
SFH <10th centile, %	64.1		

Elevated mean arterial pressure: >87 mmHg (10–18 week), >84 mmHg (18–34 week), and >86 mmHg (after week 34) (22).
High hematocrit: >41 %(1st trimester), >39 % (2nd trimester), >40% (3rd trimester) (25).

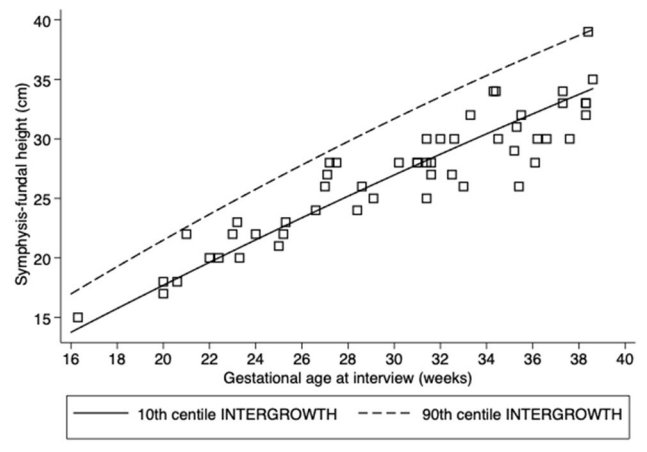

FIGURE 1 | Scatter plots of symphysis-fundal height (cm). Lines correspond to the 10 and 90th centiles according to INTERGROWTH standards.

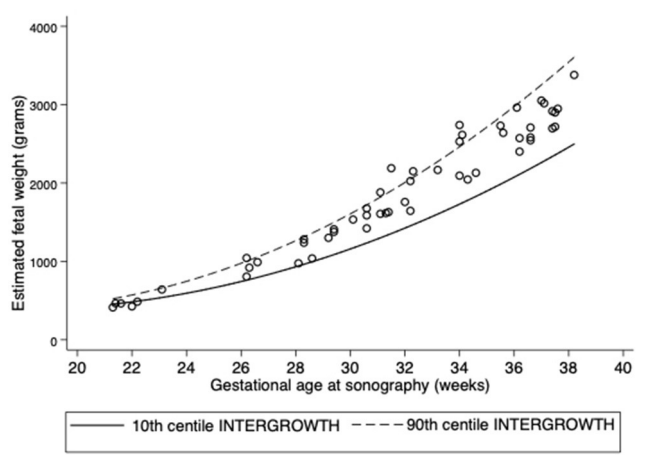

FIGURE 2 | Scatter plots of sonography-estimated fetal weight (grams). Lines correspond to the 10 and 90th centiles according to INTERGROWTH standards.

Determinants of Sonography-Estimated Fetal Weight-Z-Scores

Our final model for sonography-estimated fetal weight Z-scores, which captured 20.7% of its variability, showed that lower fetal weight was associated with low or no family support and with more hours of sleep (**Table 6**).

Amniotic Fluid Index

Multiple ordered logistic regression models for AFI (<25th, 25th−75th, and >75th centile) with elements of the adapted conflict-related stress questionnaire and the Colombian

TABLE 3 | Items and scores for (A) Adapted conflict-related stress and (B) Colombian biopsychosocial risk assessment.

A. Adapted conflict-related stress questionnaire[a]	Prevalence %	B. Colombian biopsychosocial risk score[b]	Score	Prevalence %
Recalling stressful events	49.2	**Biological risk factors**		65.6
Dreaming about stressful events	31.1	<16 years	1	3.3
Anxiety-like symptoms	42.6	>35 years	2	13.1
No interest in daily activities	36.1	First pregnancy	1	29.5
Not feeling love toward relatives and friends	31.1	>4 pregnancies	2	6.6
Easily startled	45.9	Anemia	1	4.9
Difficulty in sleeping	54.1	History of pre-term delivery	1	16.4
Stress score		History of hypertension	1	13.1
0 = stress	8.2	Current hypertension	2	3.3
>0 and ≤1: mild stress	72.1	History of pelvic surgery	1	24.6
>1 and ≤2: moderate stress	13.1	History of gestational diabetes	2	9.8
>2: severe stress	6.6	Auto-immune disease (hypothyroidism)	3	6.6
Hours of sleep		**Psychosocial risk factors**		62.3%
<3 h	4.9	Emotional tension	1 point if severe	36.0
3–6 h	16.4	Depressive mood		24.6
6–8 h	47.5	Neurovegetative symptoms[c]		9.8
>8 h	31.2	**Family support**[d]		
		No family support	1 point if absent family support[c]	6.6
		Some family support		3.3
		Moderate family support		4.9
		Strong family support		85.2
		High biopsychosocial risk	**Score ≥3**	24.6

[a] *Miller et al. (17).*
[b] *Herrera (15).*
[c] *Hands sweating, mouth dryness, blushing, pallor, tensional headache.*
[d] *Family support: mothers were asked if they were satisfied with their family or partner support, which was classified as always, sometimes or never. Reporting never resulted in a score of 1 for the general biopsychosocial risk assessment.*

TABLE 4 | Comparisons of symphysis-fundal height (SFH) Z-scores and sonography-estimated fetal weight Z-scores by binary maternal variables.

	A. Symphysis-fundal height Z-scores mean ± SD, $n = 53$			B. Sonography-estimated fetal weight mean ± SD, $n = 51$		
Condition	**yes**	**no**	***P***	**yes**	**no**	***P***
History of displacement	−2.1 ± 0.3 ($n = 15$)	−1.3 ± 0.2 ($n = 38$)	0.011	−0.21 ± 0.21 ($n = 14$)	0.17 ± 0.13 ($n = 37$)	0.065
Rural household	−1.71 ± 0.44 ($n = 10$)	−1.48 ± 0.17 ($n = 43$)	0.29	−0.36 ± 0.27 ($n = 10$)	0.17 ± 0.12 ($n = 41$)	0.031
Low income	−1.53 ± 0.23 ($n = 33$)	−1.52 ± 0.20 ($n = 20$)	0.48	−0.07 ± 0.15 ($n = 33$)	0.32 ± 0.14 ($n = 18$)	0.047
Iron supplement	−1.27 ± 0.2 ($n = 34$)	−1.98 ± 0.26 ($n = 19$)	0.016	0.04 ± 0.16 ($n = 31$)	0.11 ± 0.14 ($n = 20$)	0.37
Calcium supplement	−1.30 ± 0.19 ($n = 35$)	−1.95 ± 0.27 ($n = 18$)	0.027	0.03 ± 0.15 ($n = 32$)	0.12 ± 0.15 ($n = 19$)	0.35
Multivitamins	−1.92 ± 0.23 ($n = 21$)	−1.26 ± 0.20 ($n = 32$)	0.021	0.05 ± 0.16 ($n = 20$)	0.08 ± 0.15 ($n = 31$)	0.45
Sleeps >8 h/day	−1.37 ± 0.26 ($n = 14$)	−1.53 ± 0.19 ($n = 39$)	0.33	−0.39 ± 0.22 ($n = 14$)	0.24 ± 0.12 ($n = 37$)	0.005
Sleeps <6 h/day	−1.21 ± 0.45 ($n = 12$)	−1.62 ± 0.16 ($n = 41$)	0.14	0.44 ± 0.22 ($n = 13$)	−0.06 ± 0.13 ($n = 38$)	0.027

biopsychosocial risk assessment are described in **Table 7**. Severe lack of interest in daily activities was associated with increased odds of a larger AFI [odds ratio (OR) 8.44, 95% CI: 1.29, 54.9]. A second model for AFI showed that low family support was also associated with an increased odds of a larger AFI (OR: 9.05, 95% CI: 1.18, 69.2) (**Figure S1**), whereas the intake of folic acid supplements was associated with lower odds (OR: 0.30, 95% CI: 0.09, 0.99) (**Table 7**).

DISCUSSION

The impact of conflict on pregnancy outcomes is difficult to measure due to its intrusion in all aspects of society including antenatal care. We had the opportunity address associations of conflict-related stress with maternal-fetal outcomes including SFH which, in remote areas, is the only available tool for health professionals. Moreover, given that some women reached a

TABLE 5 | Multiple linear regression for Symphysis-fundal height (SFH) Z-scores, with (A) mean arterial pressure (MAP) and hemoglobin (grams/dL) and (B) diastolic blood pressure (DBP) and hematocrit (%).

A. SFH Z-score[a,c]	**Non-standardized Coef. ± SE**	***P***	**95% CI**	**Standardized β-coef.**	**Overall model**
History of displacement	−1.01 ± 0.31	0.001	−1.72, −0.48	−0.36	$P = 0.0010$ Adj. $R^2 = 0.262$
MAP, centile[b]	0.49 ± 0.20	0.017	0.09, 0.89	0.30	
Hemoglobin, grams/dL	0.36 ± 0.21	0.096	−0.07, 0.79	0.21	
Taking iron supplements (0 = no, 1 = yes)	0.54 ± 0.29	0.073	−0.05, 1.39	0.22	
Constant	−3.40 ± 0.62	<0.0001	−4.65, −2.14		
B. SFH Z-score[a,c]	**Non-standardized Coef. ± SE**	***P***	**95% CI**	**Standardized β-coef.**	**Overall model**
History of displacement	−1.09 ± 0.31	0.001	−1.72, −0.48	−0.41	$P < 0.0001$ Adj. $R^2 = 0.379$
Taking iron supplements (0 = no, 1 = yes)	0.59 ± 0.28	0.041	0.02, 1.15	0.24	
Hematocrit, centile	0.47 ± 0.18	0.014	0.10, 0.84	0.29	
Diastolic blood pressure, centile[b]	0.91 ± 0.25	0.001	0.39, 1.43	0.41	
Constant	−4.37 ± 0.65	<0.0001	−5.68, −3.06		

[a] *Variables that were included but were not retained in the final models (P > 0.10): # sleep hours (score), eating nuts/avocado at least once/week (0 = no, 1 = yes), history of hypertensive disorders of pregnancy (0 = no, 1 = yes).*
Model A: n = 52. VIF = 1.02. Condition number: 10.63.
Model B: n = 50. VIF = 1.03. Condition number: 12.41.
[b] *25th and 75th centiles of MAP, DBP, hemoglobin and hematocrit were calculated from our population and used instead of continuous variables in order correct for non-linear associations: MAP: <73, between 73 and 83 and >83 mmHg, DBP: <60, between 60 and 70 and >70 mmHg; hemoglobin: <12, ≥12 and ≤13.7, >13.7 grams/dL; hematocrit: <35.7, ≥35.7 and ≤41.2, >41.2%.*
[c] *After 50 bootstrap replications of linear regression models we observed that percentile- and bias-corrected confidence intervals were similar (**Table S3**).*

TABLE 6 | Multiple linear regression model for sonography-estimated fetal weight Z scores.

Fetal weight Z–score[a]	**Non–standardized Coef. ± SE**	***P***	**95% CI**	**Standardized β-coef.**	**Overall model**
Hours of sleep category[b]	−0.33 ± 0.13	0.011	−0.59, −0.08	−0.352	$P = 0.0029$ Adj. $R^2 = 0.207$
Low or no family support[c]	−0.75 ± 0.32	0.025	−1.40, −0.10	−0.302	
Weekly intake of legumes (score)[d]	−0.12 ± 0.07	0.097	−0.25, 0.02	−0.231	
Constant	1.21 ± 0.32	<0.0001	0.57, 1.85		

[a] *Variables that were included but were not retained in the final model (P > 0.10): Not stable union, rural household, aspirin intake (0 = no, 1 = yes), low income, presence of food insecurity (0 = no, 1 = yes). After 50 bootstrap replications of linear regression models we observed that percentile- and bias-corrected confidence intervals were similar (**Table S3**). n = 51. VIF = 1.12. Condition number: 6.98.*
[b] *Self-reported hours of sleep were categorized as: 0 if <3 h, 1 if ≥3 h and <6 h, 2 if ≥6 h and ≤8 h, 3 if >8 h.*
[c] *Low or no family support was categorized as: 0 = moderate or strong, 1 = low or no family support.*
[d] *Intake of legumes was categorized as: 0 = never, 1 = occasionally, 2 = 1 day/week, 3 = 2–3 days/week, 4 = >3 days/week, 5 = everyday.*

tertiary care center, sonography was also available. This allowed us to explore determinants of pregnancy outcomes for SFH, sonography-estimated fetal weight, and AFI. Several important observations emerged from this case study. First, history of displacement was an independent event that was unrelated to indicators of stress, food insecurity, and diet. Second, lower SFH Z-scores were associated with history of displacement whereas higher MAP or DBP, elevated hematocrit and iron supplementation were associated with higher SFH Z-score. Third, challenging the assumption that SFH is a proxy for fetal growth, our data showed that SFH was significantly associated with amniotic fluid index but not fetal weight. Fourth, two indicators of depression in the current pregnancy, sleeping and severe lack of interest in daily activities, were associated with lower sonography-estimated fetal weight and with higher AFI, respectively. Interestingly, these two conditions were mitigated by evidence of stronger family support. Together these results suggest that food insecurity had an indirect association with maternal and fetal outcomes, whereas different indicators of stress were directly associated with SFH, AFI, and sonography-estimated fetal weight.

History of Displacement Was Associated With Lower Symphysis-Fundal Height

Although there is evidence of an association between psychosocial stress and lower birthweight in pregnant women in the current pregnancy (36), less is known about the long-term effects of stressful situations in future pregnancies. In our

TABLE 7 | Multiple ordered logistic regression models for amniotic fluid index for elements of (A) the adapted conflict-related stress questionnaire and (B) the Colombian biopsychosocial risk assessment[a].

A. AFI and interest in daily activities[b]	**OR ± SE**	***P***	**95% CI**	**Overall model**
GA at sonography (week)	0.93 ± 0.06	0.308	0.82, 1.06	$P = 0.0359$
Lack of interest for daily activities (0 = none, 1 = mild, 2 = moderate, 3 = severe)				Pseudo $R^2 = 0.118$
Mild	2.28 ± 1.70	0.270	0.53, 9.89	
Moderate	10.59 ± 14.60	0.089	0.70, 159.21	
Severe	8.44 ± 8.07	0.026	1.29, 54.91	
Maternal age (yr)	0.93 ± 0.04	0.087	0.86, 1.01	
B. AFI and low family support[c]	**OR ± SE**	***P***	**95% CI**	**Overall model**
GA at sonography (week)	0.90 ± 0.06	0.114	0.79, 1.02	$P = 0.0112$
Folic acid supplementation (no = 0, yes = 1)	0.30 ± 0.18	0.048	0.09, 0.99	Pseudo $R^2 = 0.110$
Low or no family support[d]	9.05 ± 9.39	0.034	1.18, 69.25	

[a] *AFI, Amniotic fluid index expressed as centiles as follows: AFI <25th centile (<12.3 cm), ≤25th and ≥75th centile (12.3–16 cm) and >75th centile (>16 cm) according to our population's AFI distribution.*

[b] *Model A: n = 50. VIF = 1.03. Condition number: 12.03. Variables that were included but were not retained in the final model (P > 0.10): maternal age, taking folic acid supplements, decreased number of meals because of lack of resources, eating more eggs/week, sonography-estimated fetal weight.*

[c] *Model B: n = 50. VIF = 1.01. Condition number: 12.50. Variables that were included but were not retained in the final model (P > 0.10): taking folic acid supplements, decreased number of meals because of lack of resources, eating more eggs/week, sonography-estimated fetal weight.*

[d] *Low or no family support was categorized as: 0 = moderate or strong, 1 = low or no family support.*

population, 29.5% of the mothers had a history of displacement, which we show for the first time to be associated with lower SFH. It has been reported that symptoms of post-traumatic stress (37) are associated with adverse maternal outcomes including gestational diabetes, preeclampsia (38) and spontaneous preterm birth (39), which, respectively, were 9, 12.3, and 14% in our study population. Although women reported several symptoms included in the adapted conflict stress score, none of them were directly associated with our outcomes. Therefore, our findings would suggest that the impact of conflict-related displacement may persist but this requires further investigation.

Stress and Sonography Measurements

We found evidence that stress was associated with maternal/fetal outcomes. More severe anxiety-like symptoms and lacking interest in daily activities were associated with increased odds of a larger AFI. Large amniotic fluid volume has been associated with adverse maternal outcomes such as gestational diabetes and pregnancy-induced hypertension (28), as well as with adverse neonatal outcomes including fetal distress (40), macrosomia (41), abnormal presentation, and neonatal death (28). We showed that higher AFI values, though within normal ranges (>16 cm), were associated with maternal psychological symptoms. Thus, AFI in the highest centile may be an overlooked potential indicator of maternal/fetal distress as suggested by La Marca et al. who found an association of chronic social overload with higher concentrations of stress hormones in amniotic fluid (42). Our findings highlight the need to incorporate assessment of stress in order to identify women at risk of adverse pregnancy outcomes.

We also observed that more self-reported hours of sleep were associated with lower fetal weights. Prolonged nocturnal sleep is known to be associated with mood disorders including depression in the general population (43) and also during pregnancy (44, 45). Depression during pregnancy is a risk factor for preterm delivery and SGA (46). Moreover, we observed that lack of interest in daily activities, another symptom of depression (45), was associated with increased odds of a larger AFI. Thus, the possibility of underlying depression, suspected in women who had more hours of sleep and lack of interest in daily activities, is plausible and warrants further investigation.

Interestingly, the presence of low family support was also associated with a higher AFI and with lower sonography-estimated fetal weight. Resilience resources such as social support, personal beliefs and values, education and healthy behavioral practices have been identified as possible mitigators of stress with potential benefits on pregnancy outcomes (47). There is evidence that low social or family support is associated with impaired fetal growth (48) and that intimate social support from a partner or family member improves fetal growth (49). Both are consistent with our findings.

Indicators of Maternal Health and Well-Being

Among blood pressure measurements, higher MAP has been associated with altered placental perfusion (50) and among supplements, iron given under normal hemoglobin conditions, can lead to hemoconcentration with further increased blood viscosity, oxidative stress, and decreased perfusion of the fetus (51). On the other hand, it has also been demonstrated that an adequate perfusion measured through DBP is necessary for fetal growth (52). Interestingly, the continuous variables for DBP and MAP, as well as iron supplementation were positively associated with SFH Z-score, but were not associated with sonography-estimated fetal weight or AFI. Supporting our findings, there

is evidence that lower SFH Z-scores are associated with lower pulse pressure in a vulnerable indigenous population (53). Given the complexity of the SFH measurement, associations of blood pressure measurements with SFH warrant further investigation.

STRENGTHS AND LIMITATIONS

We acknowledge three main limitations in our study. First, we recognize that this is a cross-sectional pilot study with a small sample size. However, we were able to recruit >90% of high risk pregnancies referred to the regional hospital; only three did not attend the post-consultation interview for the project.

Second, we recognize that SFH has low sensitivity and specificity for the detection of small-for-gestational age infants, which limited our ability to identify this condition in our study population.

Third, given that we only met with women once it was difficult to explore the sensitive issue of history of displacement in depth.

Despite these limitations, an important strength of this study was the ability to identify for the first time an overlooked association of stress in a conflict zone with clinical indicators of maternal-fetal health including SFH, sonography estimated fetal weight, and AFI.

CONCLUSION

In conclusion, history of displacement and elements of the biopsychosocial stress score were associated with several quantitative biomarkers of maternal-fetal health. Our findings collectively point to the need for the integration of assessment of psychological risk factors together with measures of social and family support along with SFH and sonography measurements when identifying high-risk pregnancies to mitigate the impact of conflict-related stresses in vulnerable populations. We provide evidence that SFH may be useful in assessing maternal/fetal health when sonography is not available.

DATA AVAILABILITY STATEMENT

The datasets generated for this study may be available on written request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Scientific Research Ethics Board of San José Hospital by endorsement certificate no. 02 from April 9, 2018. The patients/participants provided their written informed consent to participate in this study as stated in Colombian Ministry of Health's Act 008430.

AUTHOR CONTRIBUTIONS

DG-F, RS, and KK wrote the initial manuscript. DG-F and RS ran statistical analyses. DG-F was responsible for inter-institutional collaborations, participated in the study design, submitted ethical approvals, interviewed participants, collected information from the files, and created the database. JC and RO, obstetricians, collected clinical data, facilitated inter-institutional collaboration with the University of Cauca, and the process of ethical approval and critically read the paper. JH facilitated interinstitutional collaborations and made important contributions to the biopsychosocial aspects of the manuscript. MS and KK participated in the study design and provided technical advice during field data collection. DG-F and KK contributed to the funding of the project. All authors read and approved the content of the article.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2020.00319/full#supplementary-material

REFERENCES

1. Garry S, Checchi F. Armed conflict and public health: into the 21st century. *J Public Health*. (2019) fdz095. doi: 10.1093/pubmed/fdz095
2. Gopalan SS, Silverwood RJ, Salman O, Howard N. Associations between acute conflict and maternal care usage in Egypt: an uncontrolled before-and-after study using demographic and health survey data. *Int J Health Policy Manage.* (2019) 8:158–67. doi: 10.15171/ijhpm.2018.107
3. Urdinola-Contreras BP. War is not healthy. Political violence and infant health outcomes in Colombia. *Rev Salud Publica.* (2018) 20:326–33. doi: 10.15446/rsap.v20n3.55430
4. McEniry M, Samper-Ternent R, Cano-Gutierrez C. Displacement due to armed conflict and violence in childhood and adulthood and its effects on older adult health: the case of the middle-income country of Colombia. *SSM Popul Health.* (2019) 7:100369. doi: 10.1016/j.ssmph.2019.100369

5. Morales JS. The impact of internal displacement on destination communities: evidence from the Colombian conflict. *J Dev Econ.* (2018) 131:132–50. doi: 10.1016/j.jdeveco.2017.10.003
6. Rivillas JC, Devia Rodriguez R, Song G, Martel A. How do we reach the girls and women who are the hardest to reach? Inequitable opportunities in reproductive and maternal health care services in armed conflict and forced displacement settings in Colombia. *PLoS ONE.* (2018) 13:e0188654. doi: 10.1371/journal.pone.0188654
7. Wanyonyi SZ, Mutiso SK. Monitoring fetal growth in settings with limited ultrasound access. *Best Pract Res Clin Obstet Gynaecol.* (2018) 49:29–36. doi: 10.1016/j.bpobgyn.2018.02.001
8. Tongprasert F, Jinpala S, Srisupandit K, Tongsong T. The rapid biophysical profile for early intrapartum fetal well-being assessment. *Int J Gynaecol Obstet.* (2006) 95:14–7. doi: 10.1016/j.ijgo.2006.05.015
9. Kim ET, Singh K, Moran A, Armbruster D, Kozuki N. Obstetric ultrasound use in low and middle income countries: a narrative review. *Reprod Health.* (2018) 15:129. doi: 10.1186/s12978-018-0571-y
10. Goto E. Prediction of low birthweight and small for gestational age from symphysis-fundal height mainly in developing countries: a meta-analysis. *J Epidemiol Community Health.* (2013) 67:999–1005. doi: 10.1136/jech-2012-202141
11. WHO Reproductive Health Library. *WHO Recommendation on Symphysis-Fundal Height Measurement*. (2018). Available online at: https://extranet.who.int/rhl/topics/preconception-pregnancy-childbirth-and-postpartum-care/antenatal-care/who-recommendation-symphysis-fundal-height-measurement (accessed January 30, 2020).
12. Fescina R H, De Mucio B, Martínez G, Alemán A, Sosa C, Mainero L, et al. *Monitoring Fetal Growth: Self-Instruction Manual*. (2011). Available online at: http://www.paho.org/Clap/index.php?option=com_docman&task=doc_details&gid=241&tmpl=component&Itemid= (accessed January 7, 2020).
13. Papageorghiou AT, Ohuma EO, Gravett MG, Hirst J, da Silveira MF, Lambert A, et al. International standards for symphysis-fundal height based on serial measurements from the fetal growth longitudinal study of the intergrowth-21st project: prospective cohort study in eight countries. *BMJ.* (2016) 355:i5662. doi: 10.1136/bmj.i5662
14. Herrera JA, Salmeron B, Hurtado H. Prenatal biopsychosocial risk assessment and low birthweight. *Soc Sci Med.* (1997) 44:1107–14. doi: 10.1016/S0277-9536(96)00240-7
15. Herrera JA. Biopsychosocial model in the prevention of maternal and perinatal morbidity and mortality. In: Editorial Program of University of Valle. 3rd ed. *[Implementation of a Biopsychosocial Model for the Reduction of Maternal and Perinatel Morbidity and Mortality in Colombia]*. Cali: Univerisity of Valle (2009). p. 15–36.
16. Ortiz Martínez RA, Castillo A. [Relationship between stress during pregnancy and spontaneous preterm delivery]. *Rev Col Psiquiatr.* (2016) 45:75–83. doi: 10.1016/j.rcp.2015.07.006
17. Miller KE, Omidian P, Quraishy AS, Quraishy N, Nasiry MN, Nasiry S, et al. The Afghan symptom checklist: a culturally grounded approach to mental health assessment in a conflict zone. *Am J Orthopsychiatry.* (2006) 76:423–33. doi: 10.1037/0002-9432.76.4.423
18. Pigott TD. A review of methods for missing data. *Educ Res Eval.* (2001) 7:353–83. doi: 10.1076/edre.7.4.353.8937
19. Little RJA. A test of missing completely at random for multivariate data with missing values. *J Am Stat Assoc.* (1988) 83:1198–202. doi: 10.1080/01621459.1988.10478722
20. Ministerio de Salud Colombia, COLCIENCIAS, Universidad Nacional de Colombia. *[Guidelines of Clinical Practice for the Prevention of Early Detection and Treatment of Pregnancy, Delivery and Postpartum Complications]*. (2013). Available online at: https://www.minsalud.gov.co/sites/rid/paginas/results.aspx?k=prevenci%C3%B3n%20embarazo&scope=Todos (accessed August 20, 2018).
21. Poon LC, Shennan A, Hyett JA, Kapur A, Hadar E, Divakar H, et al. The international federation of gynecology and obstetrics (FIGO) initiative on pre-eclampsia: a pragmatic guide for first-trimester screening and prevention. *Int J Gynaecol Obstet.* (2019) 145(Suppl. 1):1–33. doi: 10.1002/ijgo.12892
22. WHEC. *Normal Values in Pregnancy*. (2009). Available online at: http://www.womenshealthsection.com/content/obs/obs025.php3 (accessed Oct 11, 2019).
23. Ayala DE, Hermida RC. Ambulatory blood pressure monitoring for the early identification of hypertension in pregnancy. *Chronobiol Int.* (2013) 30:233–59. doi: 10.3109/07420528.2012.714687
24. WHO, CDC. *Assessing the Iron Status of Populations*. (2007). Available online at: http://apps.who.int/iris/bitstream/10665/75368/1/9789241596107_eng.pdf?ua=1&ua=1 (accessed March 23, 2016).
25. Abbassi-Ghanavati M, Greer LG, Cunningham FG. Pregnancy and laboratory studies: a reference table for clinicians. *Obstet Gynecol.* (2009) 114:1326–31. doi: 10.1097/AOG.0b013e3181c2bde8
26. Papageorghiou AT, Kennedy SH, Salomon LJ, Altman DG, Ohuma EO, Stones W, et al. The INTERGROWTH-21(st) fetal growth standards: toward the global integration of pregnancy and pediatric care. *Am J Obstet Gynecol.* (2018) 218:S630–S40. doi: 10.1016/j.ajog.2018.01.011
27. Magann EF, Sandlin AT, Ounpraseuth ST. Amniotic fluid and the clinical relevance of the sonographically estimated amniotic fluid volume: oligohydramnios. *J Ultrasound Med.* (2011) 30:1573–85. doi: 10.7863/jum.2011.30.11.1573
28. Sandlin AT, Chauhan SP, Magann EF. Clinical relevance of sonographically estimated amniotic fluid volume: polyhydramnios. *J Ultrasound Med.* (2013) 32:851–63. doi: 10.7863/ultra.32.5.851
29. Machado MR, Cecatti JG, Krupa F, Faundes A. Curve of amniotic fluid index measurements in low-risk pregnancy. *Acta Obstet Gynecol Scand.* (2007) 86:37–41. doi: 10.1080/00016340600994976
30. UNICEF, WHO. *Low Birth Weight: Country, Regional and Global Estimates*. (2004). Available online at: https://apps.who.int/iris/handle/10665/43184 (accessed February 4, 2020).
31. Sotelo N, Vásquez-Arteaga L, González-Fernández D, Marín-Agudelo N, González-Cuellar F, Montero-Carvajal J, et al. [Intestinal parasitism in preschool children from a gouvernment daycare in Popayan, Colombia]. *Med Lab.* (2017) 23:573–7. doi: 10.36384/01232576.8
32. Hackett M, Melgar-Quinonez H, Uribe MC. Internal validity of a household food security scale is consistent among diverse populations participating in a food supplement program in Colombia. *BMC Public Health.* (2008) 8:175. doi: 10.1186/1471-2458-8-175
33. ICBF, FAO. *[Colombian Guidelines Based on Foods for the Colombian Population Above 2 Years Old: Healty Dish of the Colombian Family]*. (2013). Available online at: https://www.minsalud.gov.co/sites/rid/paginas/results.aspx?k=guias%20alimentaci%C3%B3n&scope=Todos (accessed October, 2018).
34. Heinze G, Wallisch C, Dunkler D. Variable selection - a review and recommendations for the practicing statistician. *Biom J.* (2018) 60:431–49. doi: 10.1002/bimj.201700067
35. Williams R. Using the margins command to estimate and interpret adjusted predictions and marginal effects. *Stata J.* (2012) 12:308–31. doi: 10.1177/1536867X1201200209
36. Wadhwa PD, Sandman CA, Porto M, Dunkel-Schetter C, Garite TJ. The association between prenatal stress and infant birth weight and gestational age at birth: a prospective investigation. *Am J Obstet Gynecol.* (1993) 169:858–65. doi: 10.1016/0002-9378(93)90016-C
37. Greene T, Gelkopf M, Epskamp S, Fried E. Dynamic networks of ptsd symptoms during conflict. *Psychol Med.* (2018) 48:2409–17. doi: 10.1017/S0033291718000351
38. Shaw JG, Asch SM, Katon JG, Shaw KA, Kimerling R, Frayne SM, et al. Post-traumatic stress disorder and antepartum complications: a novel risk factor for gestational diabetes and preeclampsia. *Paediatr Perinat Epidemiol.* (2017) 31:185–94. doi: 10.1111/ppe.12349
39. Shaw JG, Asch SM, Kimerling R, Frayne SM, Shaw KA, Phibbs CS. Posttraumatic stress disorder and risk of spontaneous preterm birth. *Obstet Gynecol.* (2014) 124:1111–9. doi: 10.1097/AOG.0000000000000542
40. Khan S, Donnelly J. Outcome of pregnancy in women diagnosed with idiopathic polyhydramnios. *Aust NZ J Obstet Gyn.* (2017) 57:57–62. doi: 10.1111/ajo.12578
41. Morris RK, Meller CH, Tamblyn J, Malin GM, Riley RD, Kilby MD, et al. Association and prediction of amniotic fluid measurements for adverse pregnancy outcome: systematic review and meta-analysis. *BJOG.* (2014) 121:686–99. doi: 10.1111/1471-0528.12589

42. La Marca-Ghaemmaghami P, Dainese SM, Stalla G, Haller M, Zimmermann R, Ehlert U. Second-trimester amniotic fluid corticotropin-releasing hormone and urocortin in relation to maternal stress and fetal growth in human pregnancy. *Stress.* (2017) 20:231–40. doi: 10.1080/10253890.2017.1312336
43. Lopez R, Barateau L, Evangelista E, Dauvilliers Y. Depression and hypersomnia: a complex association. *Sleep Med Clin.* (2017) 12:395–405. doi: 10.1016/j.jsmc.2017.03.016
44. Tsai SY, Lee PL, Lin JW, Lee CN. Persistent and new-onset daytime sleepiness in pregnant women: a prospective observational cohort study. *Int J Nurs Stud.* (2017) 66:1–6. doi: 10.1016/j.ijnurstu.2016.11.003
45. Martínez-Paredes JF, Jácome-Pérez N. [Depression during pregnancy]. *Rev Colomb Psiquiatr.* (2019) 48:58–65. doi: 10.1016/j.rcp.2017.07.003
46. Szegda K, Markenson G, Bertone-Johnson ER, Chasan-Taber L. Depression during pregnancy: a risk factor for adverse neonatal outcomes? A critical review of the literature. *J Matern Fetal Neonatal Med.* (2014) 27:960–7. doi: 10.3109/14767058.2013.845157
47. Ramey SL, Schafer P, DeClerque JL, Lanzi RG, Hobel C, Shalowitz M, et al. The preconception stress and resiliency pathways model: a multi-level framework on maternal, paternal, and child health disparities derived by community-based participatory research. *Matern Child Health J.* (2015) 19:707–19. doi: 10.1007/s10995-014-1581-1
48. Appleton AA, Kiley K, Holdsworth EA, Schell LM. Social support during pregnancy modifies the association between maternal adverse childhood experiences and infant birth size. *Matern Child Health J.* (2019) 23:408–15. doi: 10.1007/s10995-018-02706-z
49. Hoffman S, Hatch MC. Stress, social support and pregnancy outcome: a reassessment based on recent research. *Paediatr Perinat Epidemiol.* (1996) 10:380–405. doi: 10.1111/j.1365-3016.1996.tb00063.x
50. Roberts LA, Ling HZ, Poon LC, Nicolaides KH, Kametas NA. Maternal hemodynamics, fetal biometry and doppler indices in pregnancies followed up for suspected fetal growth restriction. *Ultrasound Obstet Gynecol.* (2018) 52:507–14. doi: 10.1002/uog.19067
51. Iglesias Vazquez L, Arija V, Aranda N, Aparicio E, Serrat N, Fargas F, et al. The effectiveness of different doses of iron supplementation and the prenatal determinants of maternal iron status in pregnant Spanish women: eclipses study. *Nutrients.* (2019) 11:2418. doi: 10.3390/nu11102418
52. Mori A, Iwabuchi M, Makino T. Fetal haemodynamic changes in fetuses during fetal development evaluated by arterial pressure pulse and blood flow velocity waveforms. *BJOG.* (2000) 107:669–77. doi: 10.1111/j.1471-0528.2000.tb13311.x
53. González-Fernández D, Pons EDC, Rueda D, Sinisterra OT, Murillo E, Scott ME, et al. Identification of high-risk pregnancies in a remote setting using ambulatory blood pressure: the MINDI cohort. *Front Public Health.* (2020) 8:86. doi: 10.3389/fpubh.2020.00086

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Effect of Bushen Yutai Recipe on IVF Patients Subjected to Mild Ovarian Stimulation

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This article investigated the effects of the traditional Chinese medicine (TCM) herbal recipe, Bushen Yutai, on *in vitro* fertilization (IVF) patients subjected to mild ovarian stimulation. Two hundred nineteen infertile patients were randomly divided into 2 groups: the control group and herbal treatment group. By studying, we found estrogen levels (E_2) on the human chorionic gonadotropin (hCG) triggering day were significantly lower in the control group ($P < 0.05$), with positive blood flow being less detected by ultrasound scanning on both the day of hCG triggering and day of fresh embryo transfer for the control group ($P < 0.05$). Additionally, the blood flow index, retroactive and proactive inhibition, was higher in the control group, whereas the fertilization rate and number of high-quality embryos in the control group were lower than the control TCM experimental group ($P < 0.01$). The expression levels of the endometrial receptivity gene, vascular endothelial growth factor (VEGF), were lower in the control group vs. the TCM experimental group on the day of fresh embryo transfer ($P < 0.05$), whereas the rate of fresh embryo transfer in the control group was lower than the TCM experimental group ($P < 0.05$). In conclusion, the TCM could increase the E_2 during the IVF stage, with a higher number of oocytes and higher-quality embryos. It also improved the endometrium and increased the level of VEGF gene expression. By enhancing the fresh embryo transfer rate in a minimal ovarian stimulation protocol and by improving the clinical pregnancy and ongoing pregnancy rates, the Bushen Yutai recipe could be able to increase fresh embryo transfer and higher-quality embryos.

Keywords: endometrial receptivity, herbal, mild ovarian stimulation, traditional chinese medicine, *in vitro* fertilization and embryo transfer, herbal

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INTRODUCTION

Although significant advances have been made in the most effective and last line of treatment for infertility, *in vitro* fertilization (IVF) and embryo transfer, the implantation rate remains suboptimal (25%) (1). High-quality embryos and good endometrial receptivity are the key factors for achieving embryo implantation. Currently, with widespread clinical application of the long and short ovarian stimulation protocols, the drawbacks of these protocols are gradually being revealed (2). Ovarian hyperstimulation syndrome (OHSS) is a common complication associated with these protocols, with an occurrence rate of 10–30% (3); and clinical manifestations include pleural effusion, ascites (3), liver and kidney damage, respiratory dysfunction,

and other systemic diseases (4). Therefore, to avoid OHSS, the new mild ovarian stimulation protocols are gradually gaining popularity and being widely recognized and accepted by both doctors and patients (5). Mild ovarian stimulation protocols do not require down-regulation with gonadotropin-releasing hormone agonist (GnRHa), and the administered gonadotropin dosages are significantly reduced (6). The total dose of gonadotropin is one-third that of the standard long protocol, thereby significantly reducing the risk of ovarian hyperstimulation, post-operative bleeding, and estrogen-related symptoms. Hence, these are the major advantages of mild ovarian stimulation—less risks, less costs, and less discomfort for patients undergoing assisted reproduction treatment.

However, the use of clomiphene citrate (CC) in mild ovarian stimulation is problematic. In mild stimulation protocols, the administration of CC is routinely used to inhibit the luteinizing hormone (LH) peak and inhibit premature ovulation. But CC has an antiestrogenic effect, which could exert a detrimental effect on the endometrial receptivity. Therefore, it is often the case that most patients cannot receive fresh embryo transfer in mild ovarian stimulation cycles. Embryos have to be vitrified for a warmed-up transfer in a subsequent cycle. The risks of vitrification to long-term embryonic development and infant health are still unclear and remain a serious concern. Additional vitrified–warmed transfer procedures prolong the treatment schedule and add more costs to medical fees. As such, mild ovarian stimulation protocols are worthy of further investigations, as these aim to increase the chances of fresh embryo transfer while maintaining pregnancy rate at a considerately high level. A patient-friendly, cost-effective, and efficient mild ovarian stimulation protocol remains to be optimized. Traditional Chinese medicine (TCM) has been increasingly and widely utilized by women for the treatment of subfertility and has proven to have beneficial therapeutic effects (7). Hence in this study, the effects of Bushen Yutai recipe on the endometrial receptivity of patients undergoing the mild ovarian stimulation protocol were investigated.

MATERIALS AND METHODS

Subjects

This study was carried out in the Assisted Reproductive Center of our Hospital affiliated to Shanghai University of Traditional Chinese Medicine. Two hundred nineteen infertile patients were randomly divided into two groups (the trial registration number: ChiCTR1800016374), the experimental group with patients being administered the Bushen Yutai recipe and the control group with patients not administered the herbal recipe during mild ovarian stimulation IVF treatment. Randomization was achieved through randomized numbers generated by the SAS 9.13 software. The randomized allocation of patients was approved by the ethics committee of our hospital. All participants fulfilled the following criteria: patients were normal responders aged 25–45 years, with basic follicle-stimulating hormone levels of < 10 IU/mL and normal body mass index (18–25 kg/m^2). All patients were non-smokers and non-alcoholics. The male partners of all patients had normal semen parameters. Both husbands and wives were properly informed before signing the consent forms.

Mild Stimulation, IVF, and Embryo Transfer

Mild ovarian stimulation commenced on the third day of the menstrual cycle and included oral administration of CC (Fertilan, Cyporus) 25 mg daily, together with intramuscular injection of 150 IU human menopausal gonadotropin (Lizhu Pharmaceutical, China). When at least one follicle reached 18 mL in diameter, or when serum estradiol reached 150 pg/mL per dominant follicle, ultrasound-guided transvaginal oocyte retrieval was performed at 34–36 h after 0.1 mg of GnRHa (Diphereline, France) was subcutaneously injected together with intramuscular injection of 2,000 IU human chorionic gonadotropin (hCG) (Lizhu Pharmaceutical, China). Fertilization of the oocytes was achieved with conventional IVF. The oocytes were examined for fertilization status at 16–18 h after fertilization. Zygotes with two pronuclei were cultured for 48 h in G1 medium (Vitrolife, Sweden). Subsequently, the embryos were examined at the cleavage stage on day 3 after oocyte pickup.

On the hCG triggering day, fresh embryo transfer would be performed if the patients met the following criteria: (1)

TABLE 1 | The composition of herbal recipes.

Herbal recipes		Herbs
Bushen Yutai recipe	Bushen recipe	Prepared *rehmannia* root (15 g), *Fructus ligustri lucidi* (6 g), Semen cuscutae (6 g), *Fructus psoraleae* (9 g), *Radix ophiopogonis* (6 g)
	Yiqi recipe	*Astragalus membranaceus* (15 g), Root of hairy asiabell (9 g)
	Huoxue recipe	*Angelica slinensis* (9 g), The root of red-rooted salvia (6 g), *Rhizoma cyperi* (6 g)

TABLE 2 | Comparison of the general parameters between the two groups ($\bar{x} \pm S$).

Group	n	Average age (years)	Duration of infertility (years)	BMI (kg/m^2)
Control group	106	33.6 ± 4.0	3.2 ± 1.2	21.0 ± 1.7
TCM group	105	32.7 ± 4.6	2.9 ± 1.2	21.1 ± 1.9
P		0.1	0.1	0.9

*$P < 0.05$, the difference was statistically significant.

TABLE 3 | Comparison of basal sex hormone levels and AFC between the two groups ($\bar{x} \pm S$).

Group	n	FSH (mIU/mL)	LH (mIU/mL)	Estrodiol (pg/mL)	AFC (n)
Control group	106	6.62 ± 1.89	4.46 ± 2.30	48.08 ± 17.39	0.65 ± 0.36
TCM group	105	6.74 ± 1.64	4.82 ± 2.39	45.69 ± 14.97	0.62 ± 0.43
P		0.64	0.27	0.29	0.44

*$P < 0.05$, the difference was statistically significant.

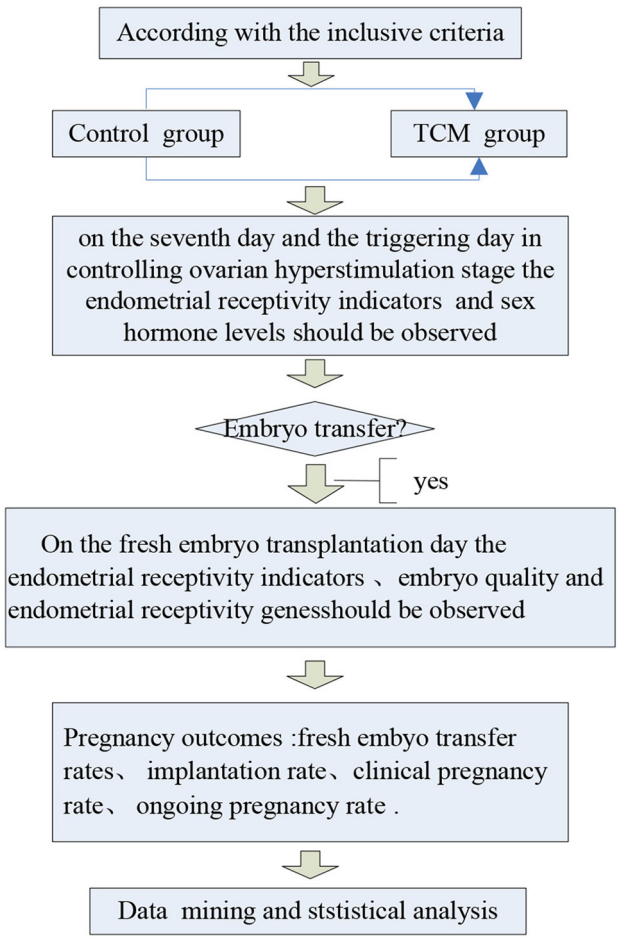

FIGURE 1 | Flowchart of the study to evaluate TCM herbs. Our doctors and researchers had explained the objectives of this study, as well as the procedure and interests of the participants.

endometrial thickness was more than 8 mm; (2) endometrium morphology pattern was A or B, and endometrial blood flow was confirmed by positive ultrasound scanning; (3) the number of high-quality embryos on day 3 was more than two; (4) patients had no risk of ovarian hyperstimulation syndrome. The vaginal progesterone gel (Crynone, Merk-serono, Germany) was utilized for luteal phase support. At 14 days after embryo transfer, pregnancy testing was performed by measuring blood hCG levels. After another additional 14 days, transvaginal ultrasound scanning was utilized to detect gestational sac and fetal heartbeat. Clinical pregnancy of patients was confirmed only if both tests were positive.

Herbal Medicine Administration

In this study, 219 patients were randomly allocated into the TCM experimental group and the control group. Eight patients withdrew from the study. The Bushen Yutai recipe was composed of 10 herbs, including Bushen components, yiqi components, and huoxue components. The herbal components are listed in **Table 1**.

TABLE 4 | Comparison of primer sequences between the two groups.

Gene markers	F-primer sequences	R-primer sequences
IL-8	TCCAAACCTTTCCACCCCAA	CCACAACCCTCTGCACCCA
IL-6	AAGCAGCAAAGAGGCACTGG	TGGCATTTGTGGTTGGGTCA
LIF	CCCAACGTGACGGACTTCCC	AGGCCTCGCAGGATGTCG
VEGF	CAGCTACTGCCATCCAATCGAG	CCTATGTGCTGGCCTTGGTG
H0XA10	ACGGCAAAGAGTGGTCGGAA	TAAAGTTGGCTGTGAGCTCCC

The average age, duration of infertility, body mass index, basal FSH level, and basic antral follicle number of patients in the control and experimental groups were comparable, as shown in **Tables 2**, **3**. A flowchart of the study is presented in **Figure 1**.

RNA Isolation, Reverse Transcription, and Real-Time Polymerase Chain Reaction

RNA was extracted or isolated by using Trizol Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Total RNA was measured by spectrophotometry at 260 nm, and 1 mg of total DNase-treated RNA per sample was used for reverse transcription by a SuperScript III kit (Invitrogen), following the manufacturer's instructions. Specific primers and probes were purchased from Applied Biosystems (Life Technologies, USA). Quantitative real-time polymerase chain reaction (qRT-PCR) analysis was performed with the 7,300 Real-Time PCR System (Applied Biosystems, USA), using SYBR Green (Life Technologies). The primer sequences of gene markers utilized for qRT-PCR analysis are shown in **Table 4**, with GAPDH (glyceraldehyde 3-phosphate dehydrogenase) being utilized as the endogenous reference control gene. The following amplification parameters were utilized for the qRT-PCR analyses: 15 min at 95°C, and 40 cycles of 10 s at 95°C, followed by 30 s at 60°C. The 2-ΔΔCt method was used to compute the relative cycle threshold (Ct) values for each gene, which were then normalized against the endogenous GAPDH gene expression. Altogether, there were three experimental replicates for each gene analyzed by qRT-PCR.

Statistical Analysis

Statistical analysis was performed using the SPSS 21 statistical software. Continuous data were presented as the mean ± SD and compared using the one-way analysis of variance test. Differences between ratios and percentage values were analyzed using the χ^2 test. $P < 0.05$ was considered to be statistically significant.

RESULTS

Comparison of Embryo Quality Between the Two Groups

The data on embryo quality in the control and experimental groups are presented in **Figure 2**. The number of oocytes in the TCM experimental group (6.2 ± 3.1) was higher than that of the control group (5.4 ± 3.4), even though the difference was not statistically significant ($P > 0.05$). The fertilization

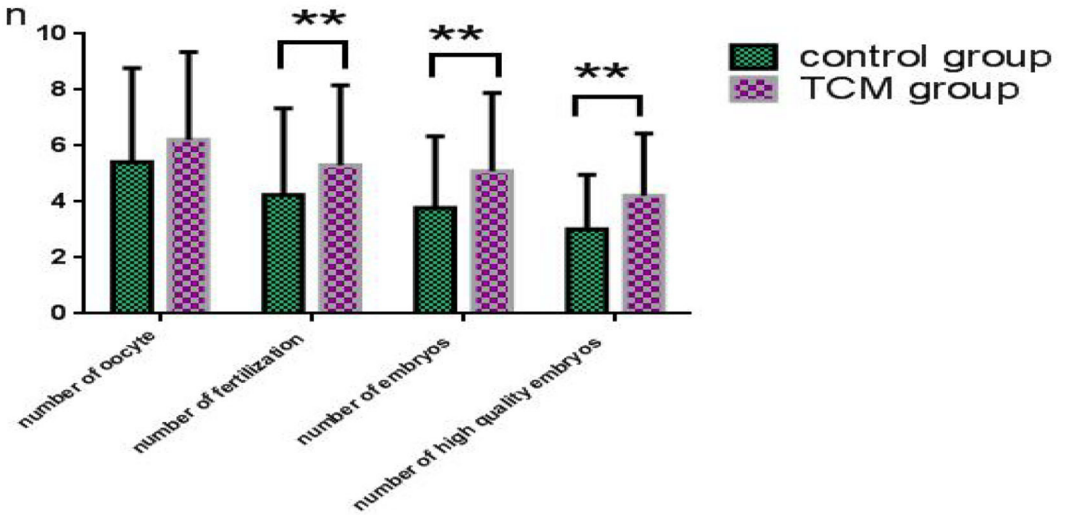

FIGURE 2 | TCM treatment yielded more good quality embryos. **Means statistically significant much.

TABLE 5 | Comparison of sex hormone levels between the two groups ($\bar{x} \pm S$) on the hCG triggering day.

Group	n	LH (mIU/mL)	Estrodiol (pg/mL)	Progesterone (ng/mL)
Control group	106	8.7 ± 6.3	2098.6 ± 1215.5	1.2 ± 0.9
TCM group	105	7.9 ± 3.8	2510.8 ± 1135.5	1.3 ± 0.8
P		0.3	0.0*	0.8

**Means Statistically significant.*

TABLE 6 | Comparison of the endometrial blood flow parameters PI and RI between the two groups ($\bar{x} \pm S$) on the hCG triggering day.

Group	n	PI	RI
Control group	106	1.4 ± 0.7	0.7 ± 0.2
TCM experimental group	105	1.1 ± 0.8	0.6 ± 0.5
P		0.0**	0.0**

***Means Statistically significant much.*

rate in the TCM experimental group was significantly higher than that of the control group, and so was the number of embryos in the TCM experimental group (5.1 ± 2.8) vs. the control group (3.8 ± 2.6) ($P < 0.01$ in both cases). Additionally, the number of high-quality embryos (4.2 ± 2.2) was also significantly higher than that of the control group (3.0 ± 2.0) ($P < 0.01$).

Comparison of Sex Hormone Levels Between the Two Groups on the hCG Triggering Day

The data on the sex hormone levels of the control and experimental groups are presented in **Table 5**. Estradiol level in the TCM experimental group (2510.8 ± 1135.5 pg/mL) was significantly higher than that of the control group (2098.6 ± 1215.5 pg/mL), ($P < 0.05$). The LH level in the TCM experimental group (7.9 ± 3.8 mIU/mL) was lower than that in the control group (8.7 ± 6.3 mIU/mL). The progesterone (P) level in the TCM experimental group (1.3 ± 0.8 ng/mL) was higher than that in the control group (1.2 ± 0.9 ng/mL), but the difference was not statistically significant ($P > 0.05$).

Comparison of Endometrial Receptivity Indicators

The data on the endometrial receptivity indicators of the control and experimental groups are presented in **Table 6** and **Figures 3**, **4**. On the hCG triggering day, the endometrial thickness of the TCM experimental group (8.7 ± 2.6 mm) was higher than that of the control group (8.3 ± 2.7 mm), but the difference was not statistically significant ($P > 0.05$). There were altogether 42 cases (39.6%) of type A endometrium and 35 cases (33.0%) of type B endometrium, and 29 cases (27.4%) of type C endometrium in the control group. Within the TCM experimental group, there were 50 cases (47.6%) of type A endometrium, 34 cases (32.4%) of type B endometrium, and 21 cases (20%) of type C endometrium. The rates of the good (type A) and the fairly good (type B) morphology endometrium was not significantly different ($P > 0.05$). However, more patients had positive endometrium blood fold under ultrasound scanning (49.5 vs. 38.7%)($P < 0.01$). In the control group, the endometrial blood flow parameters proactive inhibition (PI) (1.4 ± 0.7) and retroactive inhibition (RI) (0.7 ± 0.2) were higher than that of the TCM experimental group PI (1.1 ± 0.8) and RI (0.6 ± 0.5), and the difference was statistically significant ($P < 0.05$). Similar results were also observed with embryo transfer 3 days later.

Comparison of the Expression Levels of Endometrial Receptivity Genes Between the Two Groups on the Day of Fresh Embryo Transfer

The data on endometrial receptivity gene expression of the control and experimental groups are presented in **Table 7** and

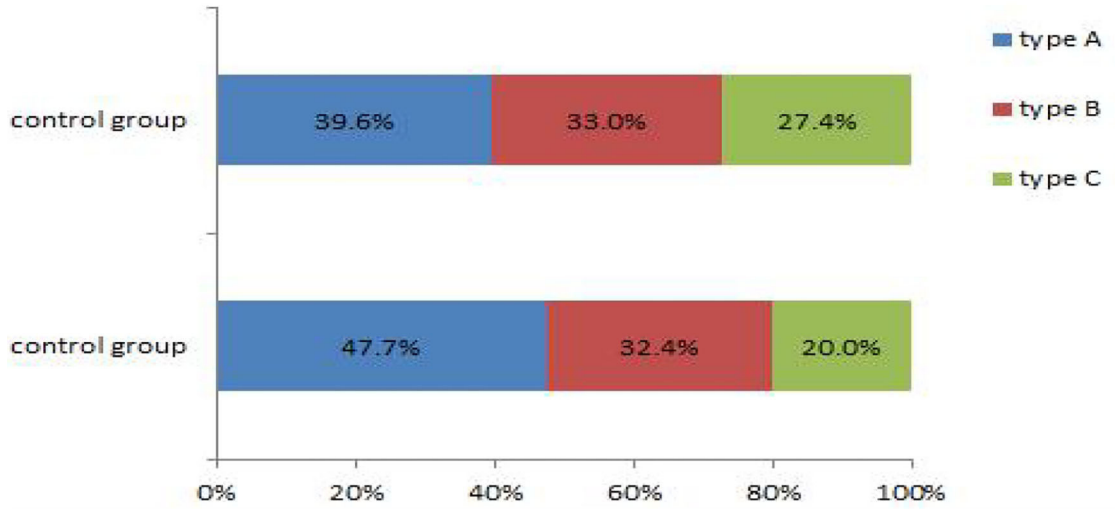

FIGURE 3 | More positive blood flow was seen in the TCM experimental group.

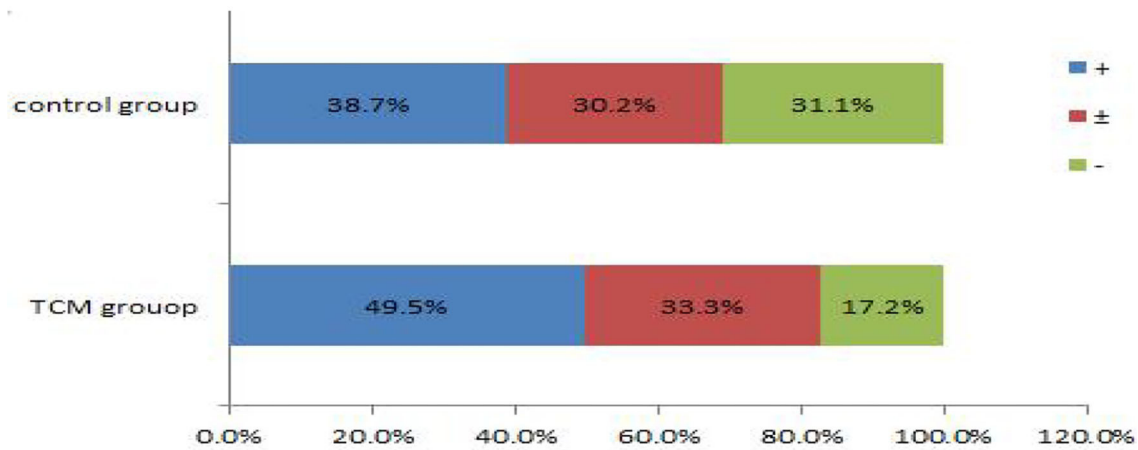

FIGURE 4 | Comparison of endometrial blood flow velocities on the hCG triggering day.

TABLE 7 | Comparison of the relative expression levels of VEGF between the two groups ($\bar{x} \pm S$).

Group	n	IL-8	IL-6	LIF	VEGF	H0XA10
Control	14	1.5 ± 1.7	1.9 ± 2.4	1.3 ± 1.0	0.8 ± 0.8	1.5 ± 1.4
TCM	22	1.1 ± 1.1	1.9 ± 2.2	1.4 ± 1.1	2.1 ± 1.9	2.0 ± 2.5
t		1.0	0.7	0.4	1.5	0.7
P		0.3	0.5	0.7	0.0*	0.5

**Means Statistically significant.*

Figure 5. The relative expression levels of IL-8, IL6, LIF, and HOXA10 were similar between the TCM experimental group and control group ($P > 0.05$). The relative expression level of the vascular endothelial growth factor (VEGF) gene in the TCM experiment group was (0.8 ± 0.8), which was significantly higher than that in the control group (2.1 ± 1.9) ($P < 0.05$).

Comparison of Pregnancy Outcomes Between the Two Groups

The data on the pregnancy outcomes of the control and experimental groups are presented in **Table 8**. Thirty-five patients (33.0%) in the control group met good endometrium criteria and therefore received fresh embryo transfer, whereas in the TCM experimental group, there were 48 patients (45.7%) with satisfactory endometrium who received fresh embryo transfer. Therefore, more patients in the TCM experimental group could receive fresh embryo transfer than the control group ($P < 0.05$). Unfortunately, there was one case in the control group and three cases in the TCM experimental group who canceled fresh embryo transfer due to catching a cold with high body temperature.

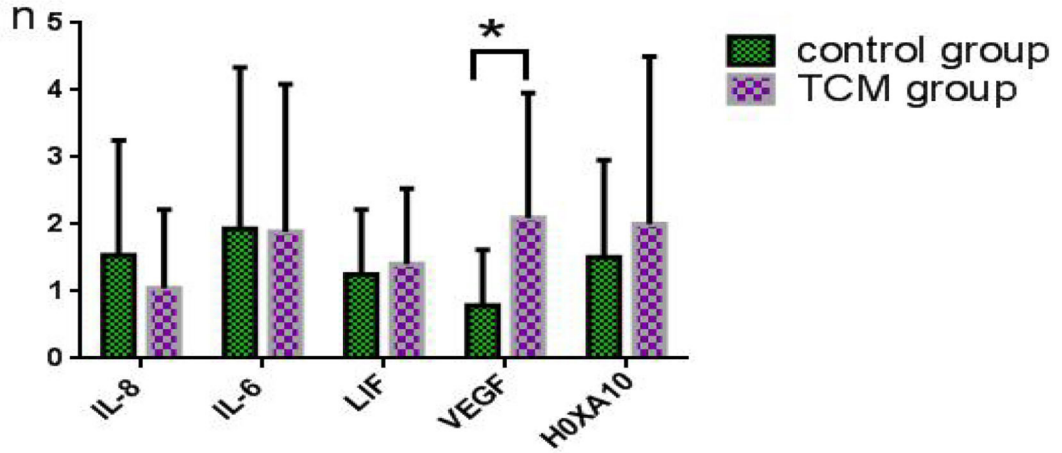

FIGURE 5 | Comparison of the expression levels of endometrial receptivity genes between the two groups on the day of fresh embryo transfer. *Means statistically significant.

Finally, 34 patients in the control group and 45 patients in the TCM experimental group received fresh embryos. The clinical pregnancy rate (38.2%) in the control group was lower than that in the TCM experimental group (40%) ($P > 0.05$). The embryo implantation rate was not significantly different either, with 20.6% for the control group vs. 24.4% for the TCM experimental group, respectively ($P > 0.05$). There were two pregnancy losses (15.4%) in the control group. However, miscarriage did not occur in the TCM experimental group.

TABLE 8 | Comparison of pregnancy outcomes between the two groups (%).

Group	n	Fresh embryo transfer rate (%)	Clinical pregnancy rate (%)	Implantation rate (%)	Miscarriage rate (%)
Control	34	35 (33.0%)	35.3% (12/34)	20.6% (14/68)	15.4% (2/12)
TCM	45	48 (45.7%)	40.0% (18/45)	24.4% (22/90)	0% (1/18)
P		0.0*	0.4	0.3	0.2

**Means Statistically significant.*

DISCUSSION

This study was a randomized controlled clinical trial to evaluate the effects of the Bushen Yutai recipe and its three components on the endometrial receptivity and ovarian responses of patients subjected to a particular mild ovarian stimulation regimen. Embryo implantation will be successful only if it occurs within a limited time period known as "the window of implantation," when the blastocyst is implantation-competent, and the endometrium is receptive (8). Additionally, several studies have shown that pregnancy will occur only when the endometrial thickness falls within a particular range of values (9, 10). On the hCG triggering day, the endometrial thickness of the TCM experimental group was higher than that of the control group, which indicated that the Bushen Yutai herbal recipe increased endometrium thickness.

As an adequate blood supply to the embryo is required for normal fetal growth, angiogenesis plays a key role during implantation (11). Dysregulated endometrial angiogenesis has been reported to lead to infertility (12). VEGF within the endometrial secretion could promote new angiogenesis within the endometrium and is known to be highly expressed during the early phase of endometrial proliferation (13). In particular, VEGF may be the most important growth factor for the development and maintenance of blood vessels (14). Our study showed that expression levels of VEGF in the TCM experimental group were significantly higher than those of the control group. VEGF could form new blood vessel networks, improve blood perfusion, and increase the implantation rate. Our data also showed that the TCM experimental group blood flow parameters PI and RI were significantly lower than those of the control group. Additionally, we also found that positive blood flow was more frequently observed in the TCM experimental group. All these thus proved that the Bushen Yutai recipe increased blood perfusion, improved the microenvironment and endometrial receptivity, and was conducive to embryo implantation.

Many TCM remedies are formulated with diverse species of herbs to increase effectiveness and reduce adverse effects through interaction with multiple molecular targets and biological pathways (15). The biological mechanisms underlying the observed beneficial effects of the Bushen Yutai recipe and its three components remain unclear. The results of this study, however, demonstrated that the positive effects might be achieved through multitarget, multisystem regulation to improve the body's endocrine levels, thereby increasing the estradiol levels and leading to increased number of oocytes and high-quality embryos. It could also involve upregulating VEGF gene expression levels in the endometrial exfoliated cells, as well as reduction of the blood flow resistance, which in turn improved the patient's endometrial receptivity. The Bushen Yutai recipe could increase the likelihood of fresh embryo transfer in patients

undergoing the mild ovarian stimulation protocol, in which fresh embryo transfer is seldom performed. This is of utmost interest to patients undergoing mild ovarian stimulation because this would decrease the number of required procedures such as embryo vitrification and warming, as well as reduce the number of hospital visits and lessen the risks of cryopreservation.

CONCLUSION

The Bushen Yutai herbal recipe improved the endometrial receptivity by increasing endometrial blood blow. For patients undergoing mild ovarian stimulation whereby fresh embryo transfer is rarely performed, this Traditional Chinese Medicine herbal formula increased the likelihood of fresh embryo transfer in a single treatment cycle.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/supplementary material.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by IRB of shuguang hospital affiliated with shanghai university of TCM. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

XJ: mainly designed experiments, clinical and experimental studies, statistical data, analysis data, and writing papers. YH: an expert in traditional Chinese medicine, has given a lot of help to the differentiation and classification of clinical Chinese medicine, especially the tongue and pulse, so that the clinical research can be carried out smoothly. ZX: the experimental research methods and steps to play a supervisory role, to ensure the accuracy of the data. TG and ZW: provided funding for the project, guided and corrected all aspects of the project, so as to complete the project.

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REFERENCES

1. de los Santos MJ, Mercader A, Galán A, Albert C, Romero JL, Pellicer A, et al. Implanta tion rates after two, three, or five days of embryo culture. *Placenta*. (2003) 24(Suppl. B):S13–9. doi: 10.1016/S0143-4004(03)00172-3
2. Makled AK, Elmekkawi SF, El-Refaie TA, El-Sherbiny MA. TAThree-dimensional power doppler and endometrial volume as predictors of malignancy in patients with postmenopausal bleeding. *Obstet Gynaecol Res*. (2013) 39:1045–51. doi: 10.1111/j.1447-0756.2012.02066.x
3. Saha PK, Goel P, Tandon R. Ovarian hyperstimulation syndrome (ohss) presentedas massive hydrothorax. *J ClinDiagn Res*. (2013) 7:2996–7. doi: 10.7860/JCDR/2013/6623.3824
4. Busso CE, Garcia-Velasco J, Gomez R, Alvarez C, Simón C, Pellicer A. Symposium: update onprediction and management of OHSS. Prevention of OHSS–dopamine agonists. *Reprod Biomed*. (2009) 19:43–51. doi: 10.1016/S1472-6483(10)60044-2
5. Mohsen IA, El Din RE. Minimal stimulation protocol using letrozole versus microdoseflare up GnRH agonist protocol in women with poor ovarian response undergoing ICSI. *Gynecol Endocrinol*. (2013) 29:105–8. doi: 10.3109/09513590.2012.730569
6. Emperaire JC. Minimal ovarian stimulation: certainly less dangerous and probably asefficient. *Gynecol ObstetFertil*. (2006) 34:354–5. doi: 10.1016/j.gyobfe.2006.02.011
7. Albertson K. *The Use of Traditional Chinese Medicine for Treating Female Infertility*. Birmingham, AL: Clayton College of Natural Health (2006)
8. Wang H, Dey SK. Roadmap to embryo implantation: clues from mouse models. *Nat Rev Genet*. (2006) 7:185–99. doi: 10.1038/nrg1808
9. Noyes N, Liu HC, Sultan K, Schattman G, Rosenwaks Z. Implantation: endometrial thickness appears to be a significant factor in embryo implantation in *in-vitro* fertilization. *Hum Reprod*. (1995) 10:919–22. doi: 10.1093/oxfordjournals.humrep.a136061
10. Barker D. Developmental origins of adult health and disease. *J Epidemiol Commun Health*. (2004) 58:114. doi: 10.1136/jech.58.2.114
11. Cui DD, Zheng CH, Gong P, Wen L, Ma WW, Zhou SC, et al. Effects of bu-shen-an-tai recipe and its two components on endometrial morphology during peri-implantation in superovulated mice. *J Huazhong Univ Sci Technol*. (2014) 35:768–74. doi: 10.1007/s11596-014-1350-9
12. Norwitz ER. Defective implantation and placentation:laying the blueprint for pregnancy complications. *Reprod Biomed Online*. (2007) 13:591–9. doi: 10.1016/s1472-6483(10)60649-9
13. Hunter RK, Nevitt CD, Gaskins JT, Keller BB, Bohler HC, LeBlanc AJ. Adipose-derived stromal vascular fraction cell effects on a rodent model of thin endometrium. *PLoS ONE*. (2015) 10:e0144823. doi: 10.1371/journal.pone.0144823
14. Sousa LM, Campos DB, Fonseca VU, Viau P, Kfoury JR, Oliveira CA, et al. Vascular endothelial growth factor A (VEGFA) modulates bovine placenta steroidogenesis *in vitro*. *Placenta*. (2012) 33:788–94. doi: 10.1016/j.placenta.2012.07.009
15. Wang L, Zhou GB, Liu P, Song JH, Liang Y, Yan XJ, et al. Dissection of mechanisms of Chinese medicinal formula Realgar-Indigo naturalis as an effective treatment for promyelocytic leukemia. *Proc Natl Acad Sci USA*. (2008) 105:4826–31. doi: 10.1073/pnas.0712365105

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Factors Influencing Vitamin D Levels in Neonatal Umbilical Cord Blood: A Two-Center Study From Tibet and Shenyang

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Objective: To investigate the factors influencing the levels of vitamin D (vitD) in the umbilical cord blood of neonates born in Naqu, Tibet (4,500 m above sea level), and Shenyang, Liaoning Province (500 m above sea level).

Methods: This prospective study was conducted from June 2017 to October 2018 in Naqu (the plateau group) and Shenyang, (the non-plateau group). Healthy mothers that gave birth to healthy neonates of >2,000g after 38 weeks' gestation were enrolled in the study, as were their neonates. After separation of serum from the umbilical cord and mothers for routine biochemical tests, discarded samples were remained for analyses of vitD, calcium, phosphorus, alkaline phosphatase (ALP) and parathyroid hormone (PTH). Questionnaires were developed covering the demographic characteristics and possible risk factors for neonatal vitD deficiency of mothers. Statistical analysis was performed to identify associations between the calcium, phosphorus, ALP, PTH, maternal factors and neonatal vitD levels.

Results: In total, 295 neonates and 225 mothers were enrolled in the study. VitD deficiency was common in neonates and mothers. The risk of vitD deficiency was higher in the plateau group than in the non-plateau group. The mean levels of 25-hydroxy vitD (25(OH)D) in mothers and neonates from the plateau group were 8.49 ± 4.12 ng/mL and 10.17 ± 5.07 ng/mL, respectively. Such levels were significantly lower than those in the non-plateau group (19.77 ± 9.57 ng/mL and 23.93 ± 11.01 ng/mL, respectively). The vitD levels of neonates and mothers were highest in the summer and lowest in the winter. Cord blood vitD was positively correlated with the vitD levels in mothers' serum ($r = 0.75$, $P < 0.05$). Increased PTH levels in mothers and decreased cord blood calcium levels were risk factors for neonatal vitD deficiency. A lack of vitD supplementation during pregnancy was associated with an 8.91-fold higher probability of neonatal vitD deficiency (OR = 8.91, 95% CI = 1.521–9.429, $P < 0.001$).

Conclusions: The levels of neonatal and maternal vitD in the plateau group were generally lower than those in the non-plateau group. VitD supplementation during pregnancy could effectively reduce the risk of vitD deficiency in neonates.

Keywords: vitD deficiency, neonates, mothers, vitD supplementation, high altitude

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INTRODUCTION

VitD is not only an important steroid hormone, but also a lipid-soluble vitamin, which has a wide range of biological effects, including regulatory effects on embryonic organ development, cell proliferation, differentiation, and maturation (1). A lack of vitD during pregnancy is the most important risk factor for infantile rickets, and may also result in poor fetal growth and neonatal development (2). Although the VitD supplementation to pregnant women is encouraged by numerous published guidelines for its prevention (3, 4), sunlight-produced VitD in the skin has played a critically important role (5) and the altitude has a dramatic influence on previtamin D_3 synthesis as well (6). When an adult wearing a bathing suit is exposed to one minimal erythemal dose of ultroviole radiation (a slight pinkness to the skin 24 h after exposure), the amount of vitamin D produced is equivalent to ingesting between 10,000 and 25,000 IU (7). Low exposure to sun, atmospheric pollution, low physical activity, indoor confinement during the day and high buildings are common risk factors of VitD deficiency in the pregnant women (8). These risk factors are infrequent at high altitudes regions as compared to plain areas. Nevertheless, VitD deficiency occurs mostly in higher altitudes (9). The prevalence of vitD deficiency during pregnancy and in neonates at high altitude (1,900–2,200 m above sea level) is higher than those in plains (10). Among nomads in Tibet (4,500 m above sea level), the 25(OH)D status is alarmingly low (11). Besides, the pregnancy alone increases the risk of vitamin D deficiency (8, 12).

There is no investigation on the vitD status among pregnant women and neonates from Tibet, the ultrahigh-altitude region. Nor have there been large-scale national survey data to report the vitD status of neonates in China. This study investigated the demographic characteristics and possible risk factors that may affect the levels of vitD, as well as tested the levels of 25(OH)D, calcium, phosphorus, ALP, and PTH in umbilical cord blood and pregnant women living in ultrahigh-altitude regions (Naqu, Tibet, 4,500 m above sea level) and the plain (Shenyang, Liaoning, 500 m above sea level). Using such data, our study aimed to fill the knowledge gap of maternal and neonatal vitD status in the ultrahigh-altitude region, as well as explore the correlation of maternal-neonatal pairs with vitD levels. Moreover, the results from regional studies are critical to develop appropriate prevention strategies during pregnancy, to optimize vitamin D status of mothers and neonates, in a region-specific manner.

METHODS AND MATERIALS

Subjects

From June 2017 to October 2018, our study recruited 225 mothers and 295 neonates delivered at the Naqu People's Hospital, Tibet (the plateau group) and the Shengjing Hospital of China Medical University, Shenyang (the non-plateau group). Healthy mothers who gave birth to healthy neonates weighing >2,000 g after 38 weeks' gestation were included, as were their neonates. Neonates with genetic metabolic diseases, infections, asphyxia, anemia, and intrauterine growth restriction, or those who were born premature were excluded from the study. Mothers with an infection during pregnancy and those who had an early membrane rupture, chorioamnionitis, preeclampsia, hypertension, gestational diabetes, chronic diarrhea, liver diseases, kidney diseases, parathyroid diseases, and other calcium-modifying conditions were also excluded. The study was approved by the Ethics Committee of the Shengjing Hospital of China Medical University (Protocol Identification Number: 2017PS33K).

Sample Collection

When the pregnant women are hospitalized for labor (within 3 days before delivery), 2 mL of venous blood for biochemical tests is taken routinely. It is difficult to collect blood from neonates, thus the samples for blood gas ion analysis and biochemical tests for neonates are from umbilical cord immediately following birth. After the separation of serum for biochemical tests, the remaining serum is retained in the test center. The samples used in our study were all discarded samples after the clinical routine diagnosis and treatment, which had no impact on the routine diagnosis and treatment of patients, and stored at −80°C until analysis at the Clinical Test Center of Shengjing Hospital. Samples from Naqu were sent to the same laboratory by the Cold-chain transportation. The gestational age, birth weight, body length, head circumference, and chest circumference of neonates were measured and recorded, as was the age, gestational times, delivery times, and the details of vitD supplementation for mothers.

Serum 25(OH)D levels were analyzed by an electrochemiluminescence immunoassay (competitive inhibition method) using the Roche E602 device. Serum PTH was also analyzed by electrochemiluminescence immunoassays (double antibody sandwich method) using the Roche E602 device. Serum calcium was detected using the methyl dimethyl phenol blue (MXB) method, phosphorus was detected using the molybdate method (UV absorption method of phosphomolybdic acid), and ALP was detected using the *P*-nitrobenzene method of phosphoric acid using with the Abbott I16200 device. Adult reference ranges are calcium (1.9–2.6 mmol/L), phosphate (1.2–1.9 mmol/L), ALP (40–375 U/L), and PTH (15–65 pg/mL).

Identification of 25(OH)D Levels

A cord blood serum 25(OH)D level <12 ng/mL (30 nmol/L) was considered as vitD deficiency, 12–20 ng/mL (30–50 nmol/L) was considered as vitD insufficiency, and >20 ng/mL (50 nmol/L) was considered as vitD sufficiency (13). In pregnant women, vitD deficiency was defined as a 25(OH)D level below 20 ng/mL (50 nmol/L), while vitD insufficiency was defined as a 25(OH)D level of 21–29 ng/mL (52.5–72.5 nmol/L) (4).

Statistical Analysis

SPSS 23.0 (SPSS, Chicago, IL, USA) was used for the processing and statistical analysis of the data. Numeric data were shown as the mean and standard deviation. Categorical data were summarized as number and percentage. The Mann-Whitney *U* test and Kruskal-Wallis test were used for statistical analyses of non-parametric data. Parametric variables were analyzed

TABLE 1 | Baseline characteristics between the plateau and non-plateau groups.

	Plateau	Non-plateau	t/χ^2-value	*P*
Neonatal BMI (kg/m^2)	12.97 ± 1.74	13.07 ± 1.53	−6.811	0.093
gestational age (week)	39.64 ± 0.87	39.78 ± 0.82	−0.628	0.531
Gender (male/female)	26/21	121/127	0.674	0.412
Medical insurance (yes/no)	45/2	238/10	0.005	0.943
Mothers age	28.85 ± 5.06	29.34 ± 4.23	−3.222	0.101
Gestational times	3.03 ± 1.48	1.72 ± 0.84	9.692	0.245
Delivery times	2.58 ± 1.12	1.57 ± 0.59	15.677	0.216

BMI for Body Mass Index.

using Student's *t*-tests and one-way analysis of variance. Unitary linear regression analysis was used to determine the association between each potential risk factor and serum 25(OH)D levels. Binary logistic regression analysis was used to calculate the odds ratios (OR) concerning vitD deficiency. A *P*-value of 0.05 was considered statistically significant.

RESULTS

General Information

There were 295 umbilical cord blood samples collected and 225 were paired with maternal samples. A total of 47 neonates (26 males; 21 females) and 47 mothers were included from the Naqu People's Hospital of Tibet, which formed the plateau group, and 248 neonates (121 males; 127 females) and 178 mothers were included from the Shengjing Hospital of China Medical University, Shenyang, Liaoning Province, which formed the non-plateau group. Among the 295 neonates, the average gestational age was 39.70 ± 0.83 weeks (range, 38–41.29 weeks), and the average birth weight was 3,319.85 ± 397.36 g (range, 2,100–4,475 g). Maternal and neonatal characteristics according to either regional group are shown in **Table 1**. There was no significant difference in the baseline characteristics between the plateau and non-plateau groups.

Among the 47 neonates in the plateau group, 39 (82.98%) exhibited 25(OH)D levels below 12 ng/mL, while seven (14.89%) exhibited 25(OH)D levels between 12–20 ng/mL, and only one (2.13%) exhibited a level above 20 ng/mL. Among the 248 neonates from the non-plateau group, 51 (20.56%) exhibited 25(OH)D levels below 12 ng/mL, while 91 (36.69%) exhibited 25(OH)D levels between 12–20 ng/mL, and 106 (42.75%) had 25(OH)D levels above 20 ng/mL. The average cord blood 25(OH)D level in the plateau group was 8.49 ± 4.12 ng/mL, which was significantly lower than that in the non-plateau group (19.77 ± 9.57 ng/mL). A statistically significant difference was observed in vitD levels from neonatal cord blood samples between the plateau and the non-plateau groups ($P < 0.05$). Among the 47 mothers in the plateau group, 44 (93.62%) exhibited 25(OH)D levels below 12 ng/mL, while three (6.38%) exhibited 25(OH)D levels between 12–20 ng/mL, and none had levels above 20 ng/mL. The average 25(OH)D level was 10.17 ± 5.07 ng/mL. As for the 178 mothers in the non-plateau group, 24 (13.48%) exhibited 25(OH)D levels below 12 ng/mL, while 53 (29.78%) exhibited 25(OH)D levels between 12–20 ng/mL, and 101 (56.74%) had 25(OH)D levels above 20 ng/mL. The average 25(OH)D level of mothers in the non-plateau group was 23.93 ± 11.01 ng/mL, which was significantly higher than that in the plateau group ($P < 0.05$; **Figure 1**, **Table 2**).

When groups were classified based on cord blood 25(OH)D levels by 20 ng/mL, categorized as deficient (<20 ng/mL) and normal (≥20 ng/mL), there were statistically significant differences in the levels of cord blood calcium, phosphorus, ALP, and PTH between the two groups, as well as in the age, gestational times, delivery times, and PTH and vitD levels among mothers ($P < 0.05$; **Table 3**). In three clinically normal neonates with very low 25(OH)D status (4.60, 4.83, and 5.18 ng/mL, respectively), their calcium, phosphate and ALP were in normal range. In one (1/295) clinically normal neonate with low 25(OH)D, 10.89 ng/mL, his calcium, phosphate and ALP (161.5U/L) were in normal range, except high PTH (91.3 pg/mL). 98.98% of the neonates had PTH lower than the normal adult range (15–65 pg/mL). However, none of the neonates had hypocalcemia. The mean values of serum calcium, phosphate, ALP, and PTH levels in mothers were within normal range. Neonates within 25(OH)D levels <20 ng/mL had higher ALP and PTH levels compared with the normal 25(OH)D group, so as their mothers.

Calcium, Phosphorus, ALP, and PTH Levels of Cord Blood in Naqu (the Plateau Group) and Shenyang (the Non-plateau Group), and Their Correlations

The levels of umbilical cord blood phosphorus and ALP were higher in the plateau group than in the non-plateau group, while birth weight was lower in the plateau group than in the non-plateau group. The differences were statistically significant ($P < 0.05$). Cord blood calcium levels were higher in the plateau group than those in the non-plateau group, while PTH levels were lower in the plateau group than in the non-plateau group. However, both differences were not statistically significant ($P > 0.05$; **Table 4**).

Correlations Between Components of the Calcium Metabolic System and VitD in Neonates

There was no linear correlation between cord blood vitD levels and cord blood PTH, phosphorus, and ALP levels ($P > 0.05$). Cord blood calcium levels were positively correlated with cord blood vitD levels, but the correlation coefficient was small ($R = 0.185$, $P < 0.01$). No linear correlation was observed between cord blood PTH and cord blood calcium, phosphorus, and ALP levels ($P > 0.05$; **Table 5**).

Correlation of VitD Metabolic System Components Between Neonates and Mothers

Cord blood vitD level was positively correlated with mothers' serum vitD level ($r = 0.75$, $P < 0.05$) and inversely weakly correlated with mothers' gestational times, and delivery times ($r = 0.175$, 0.278, respectively, $P < 0.05$ for both). The levels of

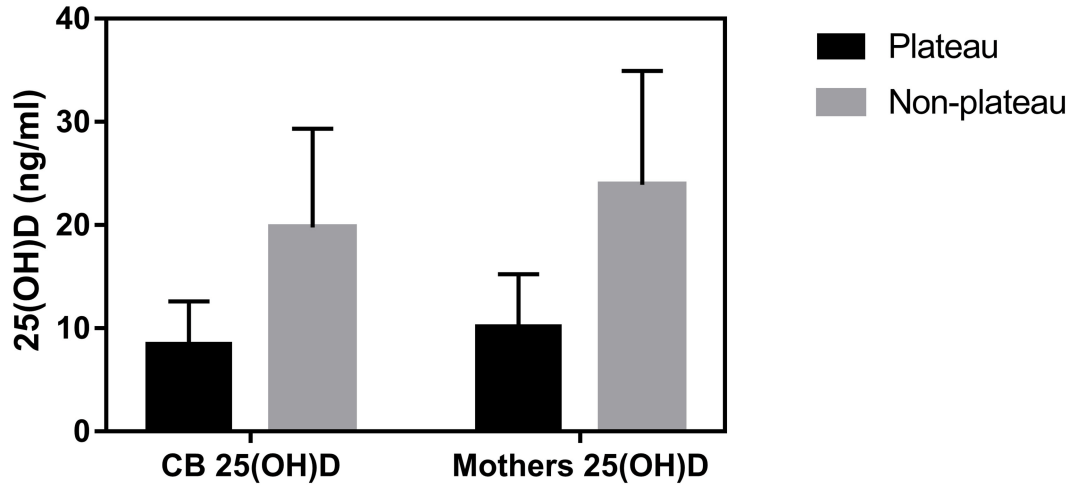

FIGURE 1 | The average 25(OH)D levels of cord blood and mothers between the plateau and non-plateau groups.

TABLE 2 | Vitamin D status of cord blood and mothers between the plateau and non-plateau groups.

	Plateau	Non-plateau
CB 25(OH)D (≤12 ng/mL) (deficient)	39 (82.90%)	51 (20.56%)
CB 25(OH)D (12–20 ng/mL) (insufficient)	7 (14.90%)	91 (36.69%)
CB 25(OH)D (≥20 ng/ml) (normal level)	1 (2.1%)	106 (42.75%)
Mothers 25(OH)D (≤20 ng/ml) (deficient)	44 (93.62%)	24 (13.48%)
Mothers 25(OH)D (20–29 ng/ml) (insufficient)	3 (6.38%)	53 (29.78%)
Mothers 25(OH)D (30–100 ng/ml) (normal level)	0	101 (56.74%)

CB for cord blood.

calcium and phosphorus in cord blood were positively weakly correlated with mothers' serum vitD level ($P < 0.05$; **Table 6**). Cord blood vitD level was positively correlated with the levels of mothers' calcium, phosphorus, but inversely correlated with the levels of mothers' ALP and PTH, both weakly, ($P < 0.05$; **Table 6**).

VitD Levels of Neonates and Mothers in Different Seasons

The blood collection time was divided into four seasons, including spring (March to May); summer (June to August); autumn (September to November); and winter (December to February). A significant seasonal difference in vitD levels was observed between cord blood and mothers' serum ($F = 3.446$ and 6.890, respectively, $P < 0.05$ for both). VitD levels in cord blood and mothers' serum were highest in the summer, with mean concentrations of 22.12 ± 7.97 ng/mL and 28.16 ± 10.47 ng/mL, respectively. Additionally, vitD levels in cord blood and mothers' serum were lowest in the winter, with mean concentrations of 17.70 ± 6.50 ng/mL and 19.47 ± 4.59 ng/mL, respectively (**Table 7**).

TABLE 3 | Univariate analysis of independent factors in neonatal cord blood vitamin D.

Variable	CB Vitamin D deficiency group (*N* = 188)	CB Normal vitamin D group (*N* = 107)	*t*	*p*
Birth weight (g)	3318.5 ± 4375.9	3322.2 ± 3164.1	−0.076	0.067
CB PTH (pg/ml)	5.16 ± 2.06	4.27 ± 2.60	−2.762	0.007
CB phosphorus (mmol/L)	1.81 ± 0.69	1.68 ± 0.29	1.823	0.028
CB calcium (mmol/L)	2.25 ± 0.42	2.35 ± 0.23	−2.215	0.001
CB ALP (U/L)	158.88 ± 70.67	146.04 ± 44.41	1.698	0.001
Mothers age	29.34 ± 4.26	29.85 ± 3.29	−1.081	0.010
Gestational times	1.90 ± 0.67	1.54 ± 0.62	2.644	<0.001
Delivery times	1.64 ± 0.51	1.18 ± 0.45	3.837	<0.001
Mothers 25(OH)D (ng/mL)	15.30 ± 7.03	29.86 ± 12.46	−10.666	<0.001
Mothers PTH (pg/mL)	23.26 ± 10.85	18.11 ± 8.37	3.339	0.001
Mothers ALP (U/L)	191.14 ± 81.67	162.81 ± 87.50	2.757	0.559
Mothers phosphorus (mmol/L)	1.20 ± 0.34	1.10 ± 0.33	2.325	0.549
Mothers calcium (mmol/L)	2.19 ± 0.14	2.19 ± 0.21	−0.035	0.136

CB for cord blood; PTH for parathyroid hormone; ALP for alkaline phosphatase.

The Correlations of Independent Factors With 25(OH)D Levels by Multivariate Analysis

There was no multicollinearity among neonatal birth weight, cord blood PTH, calcium, phosphorus, and ALP, or maternal

TABLE 4 | 25(OH)D, calcium, phosphorus, ALP, PTH of cord blood and neonatal birth weight between the plateau and non-plateau groups.

	Calcium (mmol/L)	Phosphorus (mmol/L)	ALP (U/L)	PTH (pg/mL)	Birth weight (g)
Plateau ($N = 47$)	2.34 ± 0.20	2.05 ± 0.53	212.04 ± 69.78	1.56 ± 0.95	2988.98 ± 363.75
Non-plateau ($N = 248$)	2.28 ± 0.39	1.71 ± 0.57	143.27 ± 54.86	4.82 ± 2.36	3371.34 ± 426.78
t	1.111	3.761	7.523	−4.732	−5.847
p	0.267	<0.01	<0.01	0.091	<0.01

PTH for parathyroid hormone; ALP for alkaline phosphatase.

TABLE 5 | Correlations between components of the calcium metabolic system and vitamin D in cord blood.

		25(OH)D	Calcium	Phosphorus	ALP
PTH	R	−0.003	0.106	−0.176	0.045
	P	0.964	0.069	0.052	0.446
Calcium	R	0.185			
	P	0.001			
Phosphorus	R	−0.096			
	P	0.099			
ALP	R	−0.092			
	P	0.114			

PTH for parathyroid hormone; ALP for alkaline phosphatase.

TABLE 6 | Correlation of vitamin D metabolic system components between mothers and cord blood.

	Mothers 25(OH)D	Mothers calcium	Mothers phosphorus	Mothers ALP	Mothers PTH
CB 25(OH)D R	0.75	0.134	0.141	0.127	0.221
P	<0.001	0.044	0.034	0.032	0.001

CB for cord blood; PTH for parathyroid hormone; ALP for alkaline phosphatase.

serum vitD, PTH, calcium, phosphorus and ALP levels. There was no significant difference in the effects of maternal age, gestational times, delivery times, or PTH, ALP and phosphorus levels on cord blood vitD levels by logistic regression analysis ($P > 0.05$). Decreased cord blood calcium levels and increased PTH levels in mothers were risk factors for neonatal vitD deficiency. Mothers without vitD supplementation during pregnancy were associated with an 8.91-fold higher probability of neonatal vitD deficiency at birth (OR = 8.91, 95% CI = 1.521–9.429, $P < 0.001$). The risk of neonatal vitD deficiency was 14.11-fold higher for neonates born in the plateau area, compared to neonates born in the non-plateau area (OR = 14.11, 95% CI = 1.055–7.571, $P = 0.015$).

Decreased cord blood calcium (by 1 mmol/L) was associated with a 4.69 (1/0.213)-fold higher probability of neonatal vitD deficiency at birth (OR = 0.213, 95% CI = 0.069–0.660, $P = 0.007$). The risk of cord blood vitD deficiency decreased by 13.5% for every unit of maternal vitD increase (OR = 0.881, 95% CI = 0.845–0.918, $P < 0.001$), but increased by 5.9% for every unit of maternal PTH increase (OR = 1.059, 95% CI = 1.015–1.105, $P = 0.008$; **Table 8**).

DISCUSSION

This is the first survey of vitD levels in the umbilical cord blood of neonates and their mothers in the ultrahigh altitude (4,500 m above sea level) area of Naqu, Tibet, as well as a large-scale national survey data to report the maternal and neonatal vitD status in China. Although the prevalence of vitD deficiency in mothers and neonates has been widely reported throughout the world, studies on the components of the vitD metabolism system (calcium, phosphorus, ALP, PTH) between mothers and neonates are rare. Our study included 47 pairs of mother-neonate samples from the Naqu plateau and 178 pairs of mother-neonate samples from the Shenyang non-plateau area. The level of cord blood 25(OH)D in Naqu (8.49 ± 4.12 ng/mL) was very low. It has been reported in other regions, the values of cord blood 25(OH)D were 31.0 ± 12.5 nmol/L in Zhejiang (14), 29.77 ± 12.51 nmol/L in Shanghai (15), 31.58 ± 12.72 ng/mL in Guangzhou (16) and 14.4 ± 6.7 ng/mL in Beijing (17). Vitamin D deficiency is also common in neonates and their mothers in Europe (18) and America (19). In our study, nearly half of mothers and their neonates in the non-plateau group exhibited vitD deficiency, by contrast, that prevalence increased to 100% in mothers and 97.88% in neonates residing in the plateau. The risk of vitD deficiency in neonates born in Naqu (plateau) was significantly higher than that for neonates born in Shenyang (non-plateau area). Based on previous findings (10), the geographical location at northern hemisphere and high altitude (1,900–2,200 m above sea level), might have contributed to the high prevalence of neonatal and maternal hypovitaminosis D during pregnancy. It was also found *in vitro* ampoule experiments at Everest, the production of previtamin D_3 was associated with altitude (6). Naqu is located at northern hemisphere and extremely high altitude, where the intensity of ultraviolet radiation is greater than that of the Shenyang non-plateau area. However, the annual average temperature is −2.2°C in Naqu, and the need for heavy traditional Tibetan clothing limits the body surface exposed to ultraviolet B radiation (UVB). Compared to pregnant women in the non-plateau area, the skin color of mothers in Naqu is darker, which is adverse for vitamin D synthesis. The vitD content in a traditional Tibetan diet is rare, including highland barley, wheat, beef, mutton, and ghee (11). Thus, all such factors have been

TABLE 7 | Vitamin D levels of neonates and mothers in different seasons.

Seasons	Neonates number	CB 25(OH)D	F(*P*) value	Mothers number	Mothers 25(OH)D	F(*P*) value
Spring	21	19.00 ± 7.37	F = 3.446	21	24.44 ± 9.21	F = 6.890
Summer	87	22.12 ± 7.97	*P* = 0.018	66	28.16 ± 10.47	*P* < 0.01
Autumn	84	19.29 ± 6.73		55	21.28 ± 10.07	
Winter	21	17.70 ± 6.50		19	19.47 ± 4.59	

TABLE 8 | Multivariate analysis for factors associated with vitamin D deficiency in cord blood.

Variable	B	Waldχ^2	*P*	OR	95% CI
Born in plateau	2.647	5.864	0.015	14.11	1.055–7.571
Without VitD supplementation during pregnancy	2.187	7.086	0.008	8.91	1.521–9.429
Mothers 25(OH)D	−0.127	36.355	<0.001	0.881	0.845–0.918
Mothers PTH	0.058	7.142	0.008	1.059	1.015–1.105
Mothers age	0.005	0.007	0.935	1.005	0.899–1.122
Gestational times	0.045	0.042	0.838	1.046	0.679–1.611
Delivery times	0.191	0.196	0.658	1.210	0.520–2.815
CB calcium	−1.545	7.189	0.007	0.213	0.069–0.660
CB phosphorus	1.109	3.127	0.077	3.032	0.887–10.370
CB ALP	−0.001	0.096	0.756	0.999	0.994–1.005
CB PTH	−0.077	1.172	0.279	0.926	0.806–1.064

CB for cord blood, PTH for parathyroid hormone; ALP for alkaline phosphatase.

associated with an increased prevalence of neonatal and maternal hypovitaminosis D during pregnancy (20, 21).

Our study found that neonatal vitD had a positive correlation with maternal vitD, so did neonatal calcium and phosphorus, which was consistent with cardinal features in fetal vitD metabolic system (22, 23). For neonates, maternal-fetal transport is the main source of vitD, and maternal vitD reserves during pregnancy determine the level of vitD in cord blood. Maternal hypovitaminosis D significantly increased the risk of neonatal vitD deficiency (14, 15, 18) and poor development of neonatal bone mineralization at birth, also observed in this study. The 25(OH)D concentrations in cord blood were 60–70% of their mothers' values, but optimal cord blood 25 (OH)D is not known (23, 24).

Hypovitaminosis D in many neonates may not be clinically relevant unless there is associated biochemical evidence of vitamin D deficiency (25). In this study, there was also a tendency to higher serum ALP and PTH levels in neonates with hypovitaminosis D than the values in normal-25(OH)D group. It is similar to findings in early or asymptomatic cases of vitamin D deficiency rickets (25, 26). Bone metabolic activity as measured by ALP levels was occasionally increased in patients with low 25(OH)D levels, consistent with previous studies (27–29). VitD plays an important role in the process of fetal growth, and cooperates with PTH to maintain calcium homeostasis. Long-term vitD deficiency can lead to increased PTH concentrations (30, 31). PTH is expressed in the placenta, regulates the placental expression of genes involved in the transfer of calcium and other solutes, and may directly stimulate placental calcium transfer (32). Despite many adaptations in the vitD-calcium metabolic system during pregnancy, the inverse relationship between 25(OH)D and PTH is retained, or only slightly weakened (33). In our study, neonates with hypovitaminosis D had an increase of PTH concentrations—though lower than the normal adult range, which might result from an intrauterine adaptive up-regulation of fetal PTH, as a result of maternal-fetal hypovitaminosis D, to maintain adequate calcium supply for the fetus development (34, 35). Besides, it has been previously reported that there was no correlation between 25 (OH)D and PTH in cord blood (36, 37), indicating that the well documented increase of PTH in adults with secondary hyperparathyroidism, is not evident in neonates after birth, due to temporary suppression of PTH (35). Thus, PTH levels in cord blood could be lower than the normal adult range as long as no hypocalcemia happened (35, 38), in the condition of suppression by active placental calcium transport (39). Increased PTH levels in mothers and decreased cord blood calcium levels could indicate neonatal risk of vitD deficiency. Studies on the threshold of ALP and PTH under subclinical hypovitaminosis D are limited and variable (40). Further studies are necessary to evaluate the magnitude of neonatal ALP and PTH variation under circumstances of vitamin D status at birth.

Our results demonstrated a seasonal variation in 25(OH)D. The vitD levels in cord blood and mothers were highest in summer and lowest in winter. Such findings may arise from increases in body surface exposure to sun during the summer. Seasons at sampling (autumn/winter) were reported as factors related to deficient 25(OH)D concentrations (41). Decreases in time spent outdoors, skin pigmentation, coverage and aging can all lead to insufficient vitD production (42–44). Increasing casual sun exposure for reaching the optimal serum 25(OH)D levels has been recommended (45). However, as excessive UV radiation is a carcinogen, it might be worth obtaining additional vitamin D from foods or supplements (3).

Our finding confirms that the main determinant of the vitamin D level in a neonate is the vitamin D level of the mother (46). The risk of neonatal vitD deficiency was increased almost nine-times in mothers who did not take vitD supplements during pregnancy. Strategies should be developed to prevent maternal and neonatal vitamin D deficiency. Daily prenatal vitamin D supplementation should be promoted, particularly in the 3rd trimester when the majority of placental vitamin D transfer to the fetus occurs, with the women who have insufficient sunlight

exposure taken into account (39). The current recommended intake for vitamin D during pregnancy is at least 600 IU/day of vitamin D by both the Endocrine Society (4) and the Institute of Medicine (47). And Hollis's study showed that vitamin D supplementation of 4,000 IU/day for pregnant women was safe and most effective (12). With the addition of calcium supplement in pregnancy, mothers had higher maternal concentrations of vitD (48), which had a positive effect on neonatal vitD levels (49). Supplementation of 400 IU/day (10 μg) of vitD is adequate to prevent rickets and recommended for all infants from birth to 12 months of age, independent of their mode of feeding (13).

This study had several limitations. First, maternal data that may correlate with neonatal vitD levels at birth, such as the pre-pregnancy body mass index, skin color, and sun exposure during pregnancy were not collected. However, such limitations were unlikely to affect the vitD levels of neonates at birth (50). Secondly, our analyses lacked a detailed evaluation of dietary protein and fat intake as potential food sources of vitD. Finally, the sample size from Naqu was relatively small, compared to that from Shenyang and other related studies, which may have biased the statistical analysis.

CONCLUSIONS

In conclusion, this study reported results from a prospective maternal-neonatal cohort study between the Naqu plateau and Shenyang non-plateau area, that mainly focused on neonatal vitD deficiency. The study demonstrated a high prevalence of vitD deficiency in mothers and neonates in both populations, which was purely derived from the >95% of individuals from Naqu and 50% from Shenyang who had vitD deficiency/insufficiency, and revealed the particularly higher risk of such deficiency in individuals from Naqu, Tibet. VitD supplementation should be provided to pregnant women with risk factors based on their place of residence and lifestyle, such as plateau areas. Our study also provided data on the specific-population characteristics for developing recommendations to prevent neonatal vitD deficiency in ultrahigh altitude regions.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/supplementary material.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the Shengjing Hospital of China Medical University (Protocol Identification Number: 2017PS33K). Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

INFORMED CONSENT

The study involving human participants were reviewed and approved by Shengjing Hospital of China Medical University Ethics Committee (Shenyang, China). The study does not contain patients' personal information, fully protects the patients' privacy. The samples used in the study are all discarded samples after the clinical routine diagnosis and treatment, which has no impact on the routine diagnosis and treatment of patients. The patients do not conduct additional tests/checks due to participating in the study, and there is no harm to the patients, so we got through exemption from informed consent (Protocol Identification Number: 2017PS33K).

AUTHOR CONTRIBUTIONS

MY, XL, and JL: study conception and design. MY and XL: data acquisition. MY and JL: analysis and data interpretation. MY: drafting of the manuscript. JL: critical revision. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Gluckman PD, Hanson MA, Cooper C, Thornburg KL. Effect of in utero and early-life conditions on adult health and disease. *N Engl J Med*. (2008) 359:61–73. doi: 10.1056/NEJMra0708473
2. Özdemir AA, Ercan Gündemir Y, Küçük M, Yildiran Sarici D, Elgörmüş Y, Çag Y, et al. Vitamin D deficiency in pregnant women and their infants. *J Clin Res Pediatr Endocrinol*. (2018) 10:44–50. doi: 10.4274/jcrpe.4706
3. De-Regil LM, Palacios C, Lombardo LK, Peña-Rosas JP. Vitamin D supplementation for women during pregnancy. *Cochrane Database Syst Rev*. (2016) 1:CD008873. doi: 10.1002/14651858.CD008873.pub3
4. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab*. (2011) 96:1911–30. doi: 10.1210/jc.2011-0385
5. Holick MF. Vitamin D: a D-lightful solution for health. *J Investig Med*. (2011) 59:872–80. doi: 10.2310/JIM.0b013e318214ea2d
6. Holick MF, Chen TC, Lu Z, Sauter E. Vitamin D and skin physiology: a D-lightful story. *J Bone Miner Res*. (2007) 22:V28–33. doi: 10.1359/jbmr.07s211
7. Holick MF, Chen TC. Vitamin D deficiency: a worldwide problem with health consequences. *Am J Clin Nutr*. (2008) 87:1080S–6S. doi: 10.1093/ajcn/87.4.1080S
8. Ponsonby AL, Lucas RM, Lewis S, Halliday J. Vitamin D status during pregnancy and aspects of offspring health. *Nutrients*. (2010) 2:389–407. doi: 10.3390/nu2030389
9. Looker AC, Dawson-Hughes B, Calvo MS, Gunter EW, Sahyoun NR. Serum 25-hydroxyvitamin D status of adolescents and adults in two

seasonal subpopulations from NHANES III. *Bone*. (2002) 30:771–7. doi: 10.1016/S8756-3282(02)00692-0
10. Alp H, Tekgündüz KS, Akkar MK. Maternal and cord blood vitamin D status in high-altitude pregnancy. *J Matern Fetal Neonatal Med*. (2015) 29:1–5. doi: 10.3109/14767058.2015.1011119
11. Norsang G, Ma L, Dahlback A, Zhuoma C, Tsoja W, Porojnicu A, et al. The vitamin D status among Tibetans. *Photochem Photobiol*. (2009) 85:1028–31. doi: 10.1111/j.1751-1097.2009.00552.x
12. Hollis BW, Johnson D, Hulsey TC, Ebeling M, Wagner CL. Vitamin D supplementation during pregnancy: double-blind, randomized clinical trial of safety and effectiveness. *J Bone Miner Res*. (2011) 26:2341–57. doi: 10.1002/jbmr.463
13. Munns CF, Shaw N, Kiely M, Specker BL, Thacher TD, Ozono K. Global consensus recommendations on prevention and management of nutritional rickets. *J Clin Endocrinol Metab*. (2016) 85:394–415. doi: 10.1210/jc.2015-2175
14. Zhuang XL, Zhu ZW, Zhu DB, Chen LQ, Zhao ZY, Shao J. Maternal-neonatal vitamin D status and related factors. *Chin J Pediatr*. (2012) 50:498–503.
15. Wu JY, Zhou XJ, Hong JG. Relationship of 25 (OH) D levels in cord and maternal peripheral blood. *J Clin Pediatr*. (2014) 32:1069–71.
16. Chen QZ, Gao YH, Department of Obstetrics, Guangzhou Women and Children Medical Centre. Changes of 25-hydroxy vitamin D levels in cord blood of small for gestational age and appropriate for gestational age newborns. *Chin J Woman Child Health Res*. (2016) 27:676–8.
17. Wang C, Gao J, Liu N, Yu S, Qiu L, Wang D. Maternal factors associated with neonatal vitamin D deficiency. *J Pediatr Endocrinol Metab*. (2019) 32:167–72. doi: 10.1515/jpem-2018-0422
18. Dovnik A, Mujezinović F, Treiber M, Pečovnik Balon B, Gorenjak M, Maver U, et al. Seasonal variations of vitamin D concentrations in pregnant women and neonates in Slovenia. *Eur J Obstet Gynecol*. (2014) 181:6–9. doi: 10.1016/j.ejogrb.2014.07.019
19. Merewood A, Mehta SD, Grossman X, Chen TC, Mathieu JS, Holick MF, et al. Widespread vitamin D deficiency in urban Massachusetts newborns and their mothers. *Pediatrics*. (2010) 125:640–7. doi: 10.1542/peds.2009-2158
20. Jiang L, Xu J, Pan S, Xie E, Hu Z, Shen H. High prevalence of hypovitaminosis D among pregnant women in southeast China. *Acta Paediatr*. (2012) 101:192–4. doi: 10.1111/j.1651-2227.2011.02557.x
21. Mendes MM, Darling AL, Hart KH, Morse S, Murphy RJ, Lanham-New SA. Impact of high latitude, urban living and ethnicity on 25-hydroxyvitamin D status: a need for multidisciplinary action? *J Steroid Biochem Mol Biol*. (2019) 188:95–102. doi: 10.1016/j.jsbmb.2018.12.012
22. Bhalala U, Desai M, Parekh P, Mokal R, Chheda B. Subclinical hypovitaminosis D among exclusively breastfed young infants. *Indian Pediatr*. (2007) 44:897–901.
23. Waiters B, Godel JC, Basu TK. Perinatal vitamin D and calcium status of northern Canadian mothers and their newborn infants. *J Am Coll Nutr*. (1999) 18:122–6. doi: 10.1080/07315724.1999.10718839
24. Hollis BW, Wagner CL. Assessment of dietary vitamin D requirements during pregnancy and lactation. *Am J Clin Nutr*. (2004) 79:717–26. doi: 10.1093/ajcn/79.5.717
25. Zeghoud F, Vervel C, Guillozo H, Walrant-Debray O, Boutignon H, Garabédian M. Subclinical vitamin D deficiency in neonates: definition and response to vitamin D supplements. *Am J Clin Nutr*. (1997) 65:771–8. doi: 10.1093/ajcn/65.3.771
26. Dawodu A, Agarwal M, Hossain M, Kochiyil J, Zayed R. Hypovitaminosis D and vitamin D deficiency in exclusively breast-feeding infants and their mothers in summer: a justification for vitamin D supplementation of breast-feeding infants. *J Pediatr*. (2003) 142:0–173. doi: 10.1067/mpd.2003.63
27. Maghbooli Z, Hossein-Nezhad A, Shafaei AR, Karimi F, Madani FS, Larijani B. Vitamin D status in mothers and their newborns in Iran. *BMC Pregnancy Childbirth*. (2007) 7:1–6. doi: 10.1186/1471-2393-7-1
28. Gessner BD, Plotnik J, Muth PT. 25-Hydroxyvitamin D levels among healthy children in Alaska. *J Pediatr*. (2003) 143:434–7. doi: 10.1067/S0022-3476(03)00410-4
29. Glerup H, Mikkelsen K, Poulsen L, Hass E, Overbeck S, Thomsen J, et al. Commonly recommended daily intake of vitamin D is not sufficient if sunlight exposure is limited. *J Intern Med*. (2000) 247:260–8. doi: 10.1046/j.1365-2796.2000.00595.x
30. Houghton LA, Szymlek-Gay EA, Gray AR, Ferguson EL, Deng X, Heath AL. Predictors of vitamin D status and its association with parathyroid hormone in young New Zealand children. *Am J Clin Nutr*. (2010) 92:69. doi: 10.3945/ajcn.2009.29055
31. Specker B. Vitamin D requirements during pregnancy. *Am J Clin Nutr*. (2004) 80:1740S. doi: 10.1093/ajcn/80.6.1740S
32. Simmonds CS, Karsenty G, Karaplis AC, Kovacs CS. Parathyroid hormone regulates fetal-placental mineral homeostasis. *J Bone Miner Res*. (2010) 25:594–605. doi: 10.1359/jbmr.090825
33. Kiely M, Hemmingway A, O'Callaghan, Karen M. Vitamin D in pregnancy: current perspectives and future directions. *Ther Adv Musculoskelet Dis*. (2017) 9:145–54. doi: 10.1177/1759720X17706453
34. Wagner CL, Hollis BW, Kotsa K, Fakhoury H, Karras SN. Vitamin D administration during pregnancy as prevention for pregnancy, neonatal and postnatal complications. *Rev Endocr Metab Disord*. (2017) 18:307–22. doi: 10.1007/s11154-017-9414-3
35. Karras SN, Koufakis T, Antonopoulou V, Goulis DG, Annweiler C, Pilz S, et al. Characterizing neonatal vitamin D deficiency in the modern era: a maternal-neonatal birth cohort from Southern Europe. *Steroid Biochem Mol Biol*. (2020) 198:105555. doi: 10.1016/j.jsbmb.2019.105555
36. Bowyer L, Catling-Paull C, Diamond T, Homer C, Davis G, Craig ME. Vitamin D, PTH and calcium levels in pregnant women and their neonates. *Clin Endocrinol (Oxf)*. (2008) 70:372–7. doi: 10.1111/j.1365-2265.2008.03316.x
37. Young BE, McNanley TJ, Cooper EM, McIntyre AW, Witter F, Harris ZL, et al. Maternal vitamin D status and calcium intake interact to affect fetal skeletal growth in utero in pregnant adolescents. *Am J Clin Nutr*. (2012) 95:1103–12. doi: 10.3945/ajcn.111.023861
38. Cao YM. *Study on Vitamin D Nutritional Status and Related Factors of Perinatal Women and Newborns in Suzhou*. Suzhou: Suzhou University (2013).
39. Bass JK, Chan GM. Calcium nutrition and metabolism during infancy. *Nutrition*. (2006) 22:1057–66. doi: 10.1016/j.nut.2006.05.014
40. Hatun S, Ozkan B, Orbak Z, Doneray H, Cizmecioglu F, Toprak D, et al. Vitamin D deficiency in early infancy. *J Nutr*. (2005) 135:279–82. doi: 10.1093/jn/135.2.279
41. Karras SN, Anagnostis P, Annweiler C, Naughton DP, Petroczi A, Bili E, et al. Maternal vitamin D status during pregnancy: the Mediterranean reality. *Eur J Clin Nutr*. (2014) 68:864–9. doi: 10.1038/ejcn.2014.80
42. Holvik K, Meyer HE, Haug E, Brunvand L. Prevalence and predictors of vitamin D deficiency in five immigrant groups living in Oslo, Norway: the Oslo Immigrant Health Study. *Eur J Clin Nutr*. (2005) 59:57–63. doi: 10.1038/sj.ejcn.1602033
43. Van der Meer IM, Karamali NS, Boeke AJ, Lips P, Middelkoop BJ, Verhoeven I, et al. High prevalence of vitamin D deficiency in pregnant non-Western women in The Hague, Netherlands. *Am J Clin Nutr*. (2006) 84:350–3. doi: 10.1093/ajcn/84.2.350
44. Kaushal M, Magon N. Vitamin D in pregnancy: a metabolic outlook. *Indian J Endocrinol Metab*. (2013) 17:76–82. doi: 10.4103/2230-8210.107862
45. Holick MF. Too little vitamin D in premenopausal women: why should we care? *Am J Clin Nutr*. (2002) 76:3–4. doi: 10.1093/ajcn/76.1.3
46. Halicioglu O, Aksit S, Koc F, Akman SA, Albudak E, Yaprak I, et al. Vitamin D deficiency in pregnant women and their neonates in spring time in western Turkey. *Paediatr Perinat Epidemiol*. (2012) 26:53–60. doi: 10.1111/j.1365-3016.2011.01238.x
47. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, et al. The 2011 report on dietary reference intakes for calcium and vitamin d from the institute of medicine: what clinicians need to know. *Obstet Gynecol Surv*. (2011) 66:356–7. doi: 10.1097/OGX.0b013e31822c197a
48. Park H, Brannon PM, West AA, Yan J, Jiang X, Perry CA, et al. Maternal vitamin D biomarkers are associated with maternal and fetal bone turnover among pregnant women consuming controlled

amounts of vitamin D, calcium, and phosphorus. *Bone*. (2017) 95:183–91. doi: 10.1016/j.bone.2016.12.002

49. Wang Y, Li H, Zheng M, Wu Y, Zeng T, Fu J, et al. Maternal vitamin D deficiency increases the risk of adverse neonatal outcomes in the Chinese population: a prospective cohort study. *PLoS ONE*. (2018) 13:e0195700. doi: 10.1371/journal.pone.0195700
50. Marshall I, Mehta R, Ayers C, Dhumal S, Petrova A. Prevalence and risk factors for vitamin D insufficiency and deficiency at birth and associated outcome. *BMC Pediatr*. (2016) 16:208. doi: 10.1186/s12887-016-0741-4

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Management of Cervical Cancer in Pregnant Women: A Multi-Center Retrospective Study in China

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Background: This retrospective multi-center study aimed to describe the epidemiological characteristics, clinical features, and management of patients with cervical cancer in pregnancy (CCIP) and evaluate maternal and infant outcomes.

Methods: The data of patients with CCIP were retrospectively collected from those diagnosed and treated in 17 hospitals in 12 provinces in China between January 2009 and November 2017. The information retrieved included patients' age, clinical features of the tumor, medical management (during pregnancy or postpartum), obstetrical indicators (i.e., gestational age at diagnosis, delivery mode, and birth weight), and maternal and neonatal outcomes. Survival analyses were performed using Kaplan–Meier survival curves and log-rank tests that estimated the overall survival of patients.

Results: One-hundred and five women diagnosed with CCIP (median age = 35 years) were identified from ∼45,600 cervical cancer patients (0.23%) and 525,000 pregnant women (0.020%). The median gestational age at cancer diagnosis was 20.0 weeks. The clinical-stage of 93.3% of the patients with CCIP was IB1, 81.9% visited the clinic because of vaginal bleeding during pregnancy, and 72.4% had not been screened for cervical cancer in more than 5 years. To analyze cancer treatments during pregnancy, patients were grouped into two groups, termination of pregnancy (TOP, $n = 67$) and continuation of pregnancy (COP, $n = 38$). Analyses suggested that the TOP group was more likely to be diagnosed at an earlier gestational stage than the COP group

(14.8 vs. 30.8 weeks, $p < 0.001$). The unadjusted hazard ratio for the COP group's overall survival was 1.063 times that of the TOP group (95% confidence interval = 0.24, 4.71). There were no significant differences between the TOP and COP groups in maternal survival ($p = 0.964$). Thirty-three of the infants of patients with CCIP were healthy at the end of the follow-up period, with a median age of 18 ± 2.8 months.

Conclusions: Most patients with CCIP had not been screened for cervical cancer in over 5 years. The oncologic outcomes of the TOP and COP groups were similar. A platinum-based neoadjuvant chemotherapy regimen could be a favorable choice for the management of CCIP during the second and third trimesters of pregnancy.

Keywords: continuation, cervical cancer, pregnancy, neoadjuvant chemotherapy, termination

INTRODUCTION

China accounts for ∼1/5th of the world's population. Cervical cancer is a common malignant tumor that seriously threatens the health of Chinese women. According to the National Cancer Report 2015, 98,900 cervical cancer cases were newly reported that year, causing 30,500 new deaths in China (1). The Chinese government has implemented several programs to control cervical cancer, including the National Cervical Cancer Screening Program in Rural Areas, which started in 2009. Due to variability in medical resources among regions, cervical cancer screening rates vary highly across regions, especially in rural and medically underserved areas (2, 3).

Cervical cancer in pregnancy (CCIP) is a rare event, which occurs in ∼0.004–0.1% of pregnant and postpartum women (4, 5). The variation in the incidence of cervical cancer during pregnancy is likely to reflect the differences in underlying cervical cancer incidence across the population and screening programs (6). The management of CCIP is challenging and the rare nature of such conditions has resulted in a lack of reference data from randomized studies or large trials. Thus, the guidelines for the management of CCIP are currently based on limited data from a small number of cases and expert opinions (6–8). Since both maternal and fetal benefits need to be taken into consideration in the management of CCIP, it is imperative to provide an individualized approach and psychological support throughout pregnancy, and treatment decisions should be made by collaborative and multidisciplinary teams consisting of gynecologic oncologists, obstetricians, pathologists, and neonatologists (9).

In recent years, treatment for CCIP has gradually shifted from aggressive therapies to more pregnancy-preservative management, particularly for patients in the early stages of cervical cancer within the second or third gestational trimester (10). Fertility-preservative options that include radical or simple trachelectomy with or without neoadjuvant chemotherapy (NACT) have been successfully applied in cervical cancer patients (11). Surgery may be proposed as the primary treatment for early-stage cervical cancer (10). In contrast, NACT is an optional treatment for patients with advanced-stages of cervical cancer, which may postpone the definitive local treatment until term or after delivery (10).

Given the limited data on maternal and fetal prognosis (i.e., continuation vs. termination of pregnancy), our study aimed to contribute to clinical evidence by assessing the clinical characteristics, management, and prognosis of cervical cancer in pregnant women with different gestational age (GA) and compare the subsequent outcomes of termination of pregnancy (TOP) and continuation of pregnancy (COP).

MATERIALS AND METHODS

This is a hospital-based retrospective descriptive study, in which patients who were diagnosed with CCIP from January 2009 to November 2017 (data retrieving period) were selected. Data on patients were collected from their medical records, which were kept in the archives of 17 hospitals located in 12 cities. These hospitals included the Peking University People's Hospital, Peking University First Hospital, Beijing Chaoyang Hospital, Capital Medical University and Beijing Tsinghua Changgung Hospital in Beijing; The Obstetrics & Gynecology Hospital of Fudan University in Shanghai; Qilu Hospital of Shandong University in Jinan; West China Second University Hospital of Sichuan University in Chengdu; The First Affiliated Hospital of Zhengzhou University in Zhengzhou; Jiangxi Maternal and Child Health Hospital in Nanchang; Guizhou Provincial People's Hospital in Guiyang; The First Affiliated Hospital of Xi'an Jiaotong University in Xi'an; The First Affiliated Hospital of Nanjing Medical University and Nanjing Drum Tower Hospital in Nanjing; The Second Affiliated Hospital of Chongqing Medical University and Chongqing University Cancer Hospital in Chongqing; ShengJing Hospital of China Medical University in Shenyang; and Hainan General Hospital in Haikou.

The study was approved by the lead hospital's (i.e., Peking University People's Hospital's) Institutional Review Board (PKUPH IRB, 2018PHB230-01), and all other 16 hospitals provided their IRBs' comments and agreed to follow the terms laid out by the lead hospital's IRB. Prior to their treatment, the enrolled patients' treatments were performed according to protocols that were decided by multidisciplinary teams, and patients provided informed consent, permitting their medical records (from diagnosis to follow-up) to be

used for further analysis. As a result, the IRB approved the retrospective study without the need to obtain further written informed consent from patients. However, we still prepared an informed consent form for patients to sign by mail in case, (1) any patient requested to be removed from the study, and (2), in cases when an informed consent form signed by a patient from any subsite in the treatment stage did not clearly state that they permitted their data to be used for further research.

Patients were included in the analyses if they were diagnosed with CCIP during gestation, according to the FIGO (International Federation of Gynecology and Obstetrics) 2009 instructions for cervical cancer staging (12) and World Health Organization (WHO) criteria for histopathological diagnosis of cervical cancer, and had complete records (i.e., data) from diagnosis to follow-up.

The retrospective analysis was performed on the following parameters: each patient's basic information (i.e., age and gestational age, GA at diagnosis), tumor features (i.e., FIGO staging and histopathology), cancer management (during pregnancy or postpartum), patient response to treatment (as measured by results from clinical examinations and magnetic resonance imaging, MRI, or ultrasonography), obstetrical indicators (i.e., obstetric history, mode and GA of delivery, birth weight, 5-min Apgar scores, and neonatal outcome), and maternal outcome.

For analyses, patients were categorized into the TOP group if fetal preservation was abandoned and the COP group if the fetus was kept according to the patient's wishes. Treatment options encompassed by COP included a (1) surgery sub-group: surgical treatments including conization/radical trachelectomy ± lymphadenectomy, (2) NACT sub-group: NACT administration during pregnancy, and (3) post-delivery treatment (PDT) sub-group: regular follow-up for the tumor without definitive treatment until delivery. Termination of pregnancy refers to feticide treatment options such as hysterectomy or chemoradiotherapy with the fetus *in utero* or after a previous evacuation and are usually performed during the first or second trimesters. To analyze the overall survival, deaths from any cause by March 12, 2018 (the endpoint of the follow-up) were recorded. Patients who did not experience cancer recurrence or were alive at the end of the follow-up were censored at the last known date before or at the end of follow-up. The physical and cognitive development statuses of the children of the COP group were acquired during the follow-up.

Statistical Analysis

Descriptive statistics were calculated for tumor features and obstetrical indicators. Categorical data were summarized using frequencies and percentages, and continuous data were summarized using means and standard deviations (SD) for normally distributed data and medians and ranges for non-normally distributed data. Clinical characteristics were compared between TOP and COP groups using 95% confidence intervals (95% CIs) for differences in means or proportions, as appropriate. The survival rate was compared using the Kaplan-Meier method, a survival curve was drawn, and log-rank tests were used to assess differences. SPSS 16.0 software (SPSS Inc., Chicago, IL, USA) was used for data processing and analysis. $P < 0.05$ was considered to indicate a statistically significant difference.

RESULTS

Patient Characteristics at Diagnosis

A total of 105 women diagnosed with CCIP were identified from 45,600 patients with invasive cervical cancer (0.23%, 105/45,600). This represented 0.020% of the 525,000 pregnant women (105/525,000) who presented at the site hospitals during the same period. The median age at diagnosis of patients with CCIP was 35.0 ± 5.3 years (17–45 years). Thirty-eight (36.2%) of those patients were diagnosed in the first trimester of gestation, 33.3% in the second, and 30.5% in the third. Ninety-eight (93.3%) of patients with CCIP were histologically staged at level IB1 and above at the primary diagnosis. Squamous carcinoma (SCC) and adenocarcinoma (AC) accounted for 94.3% (99/105) of all the histology. Pregnancy was terminated in 63.8% (67/105) of patients (the TOP group), while pregnancy was continued in 36.2% (38/105) of the patients (the COP group). The median GA at diagnosis in the TOP group was 14.8 weeks (5–31weeks) and 30.8 weeks (6–41 weeks) in the COP group ($P < 0.01$).

Abnormal bleeding during pregnancy was the main symptom in 85.7% (90/105) of the patients. Notably, only five (4.76%) of the 105 included patients were screened for cervical cancer within the 5 years that preceded the diagnosis, and 76 (72.4%) of the patients had not been screened for cervical cancer for over 5 years at the time of diagnosis. Among the 24 (22.9%) patients screened during or before their pregnancies with cytology and/or HPV testing, 17 were tested due to cervical cancer-related symptoms, and seven were screened with cytology without showing any carcinoma-related abnormalities in their medical histories or physical examinations. Of the seven patients screened with cytology, four had ASC-US cytology, and three had HSIL cytology. They were diagnosed with CCIP due to abnormal biopsies. Except for the differences in stage IB2 and GA at diagnosis, there were no significant differences between the TOP and COP groups in patients' age, gravidity, parity, FIGO staging, and tumor size (**Table 1**).

Management During Pregnancy

In the COP group, cervical surgeries were only performed in three patients (surgery sub-group) who were diagnosed early, at an average of 18 weeks of GA and staged ≤ Ib2 (**Table 2**). Of the three patients, one patient with a stage IA2 tumor underwent conization at 12 weeks GA, and two patients with stage IB1 tumors underwent radical trachelectomy at 26 and 18.6 weeks of gestation, respectively. Among the two patients with stage IB1 cancer, the one who underwent radical trachelectomy at 26 weeks of GA also underwent lymphadenectomy. The other patient did not undergo lymphadenectomy but was treated with concurrent chemoradiotherapy (CCRT) after delivery, experienced cancer

TABLE 1 | Descriptive statistics of demographic and tumor characteristics ($n = 105$).

	Total ($n = 105$)	TOP ($n = 67$)	COP ($n = 38$)	Difference in means (95% CI)
Age (yrs), mean (SD), range	35.0 (5.3), 17–45	34.2 (5.1), 17–44	32.3 (5.6), 23–45	1.8 (−0.2 to 4.0)
Gravida, mean (SD), range	3.6 (1.7), 1–9	3.8 (1.6), 1–8	3.2 (1.9), 1–9	0.6 (−0.4 to 1.3)
Para, mean (SD), range	1.3 (1.2), 0–7	1.4 (1.0), 0–5	1.3 (1.5), 0–7	0.1 (−0.3 to 0.6)
GA at diagnosis (wks), mean (SD), range	20.0 (11.1), 5–41	14.8 (7.7), 5–31	30.8 (8.5), 6–41	−16.0 (−19.3 to −12.8)*
Method of disease detection, *n* (%)				
Bleeding	90 (85.7)	57 (85.1)	33 (86.8)	−1.8 (−14.6 to 13.9)
Physical examination	8 (7.6)	4 (6.0)	4 (10.5)	−4.6 (−18.6 to 6.0)
Abnormal cervical cancer screening	7 (6.7)	6 (8.9)	1 (2.6)	6.3 (−5.6 to 15.8)
FIGO Stage, *n* (%)				
IA	7 (6.7)	3 (4.5)	4 (10.5)	−6.1 (−20.0 to 4.1)
IB1	30 (28.6)	19 (28.4)	11 (28.9)	−0.6 (−19.0 to 16.2)
IB2	33 (31.4)	26 (38.8)	7 (18.4)	20.4 (1.9 to 35.5)*
II	28 (26.6)	17 (25.4)	11 (28.9)	−3.6 (−21.7 to 13.1)
III–IV	7 (6.7)	2 (3.0)	5 (13.2)	−10.2 (−24.5 to 0.2)
Pathological type, *n* (%)				
Squamous cell carcinoma	83 (79.1)	53 (79.1)	30 (78.9)	0.16 (−14.8 to 17.4)
Adenocarcinoma	16 (15.2)	10 (14.9)	6 (15.8)	−0.9 (−16.9 to 12.5)
Other type†	6 (5.7)	4 (6.0)	2 (5.3)	0.7 (−11.8 to 9.9)
Tumor size, *n* (%)				
≤4 cm	46 (43.8)	30 (44.8)	16 (42.1)	2.7 (−16.7 to 21.2)
>4 cm	59 (56.2)	37 (55.2)	22 (57.9)	−2.7 (−21.2 to 16.7)

TOP, termination of pregnancy; COP, continuation of pregnancy; GA, gestational age; yrs, years; wks, weeks.
**P < 0.01.*
† Three cases of small cell carcinoma, one case of poorly differentiated carcinoma, one case of cervical sarcoma, and one case of neuroendocrine carcinoma.

TABLE 2 | Characteristics of patients in the COP group, stratified by management modality ($n = 38$).

	Surgery sub-group ($n = 3$)	NACT sub-group ($n = 10$)	PDT sub-group ($n = 25$)
Age (years)	35 (25-36)	33 (23-37)	34 (23-45)
GA at diagnosis (weeks)	18(6-25)	26 (19-32)	37 (9-41)
FIGO Stage, *n* (%)			
≤IB1	3 (20)	2 (13.3)	10 (66.7)
>IB1	0 (0)	8 (34.8)	15 (65.2)
Pathological type, *n* (%)			
Squamous cell carcinoma	2 (6.7)	8 (26.7)	20 (66.7)
Non–squamous cell carcinoma	1 (12.5)	2 (25)	5 (62.5)
Tumor size, *n* (%)			
≤4 cm	3 (18.8)	2 (12.5)	11 (68.8)
<4 cm	0 (0)	8 (36.4)	14 (63.6)
GA at delivery (weeks)	36 (34–38)	34 (30–35)	37 (28–41)
Delivery weight (grams)	3,290 (1,700–3,320)	2,352 (1,350–2,610)	2,980 (1,315–4,050)
Mother's outcome, *n* (%)			
DOD	1 (33.3)	0 (0)	2 (66.7)
NED	2 (6.5)	10 (32.3)	19 (61.3)*

DOD, dead of disease; NED, no evidence of disease; ND, not determined; GA, gestational age; NACT, neoadjuvant chemotherapy; PDT, post-delivery treatment.
**Another four cases were lost to follow-up.*

recurrence, and died within the 5-year follow-up. Platinum-based neoadjuvant chemotherapy was administered to 10 (26.3%) pregnant patients (the NACT sub-group) during the second or third trimester, at 26 (range 19–32) weeks of gestation on average. Eighty percent (8/10) of those patients had stage IB2 tumors $\geq$ 4 cm in size. Eighty percent (8/10) of the tumors in those patients were also classified as squamous cell carcinoma (**Table 2**). Cisplatin combination therapy was administered to all ten patients, of which eight received cisplatin plus paclitaxel, one received cisplatin plus vincristine and bleomycin, and one received cisplatin plus bleomycin and etoposide. On average, 3.5 cycles of chemotherapy were administered during pregnancy in dosages similar to those for non-pregnant patients, with adjustments made for patients' actual weight, height, and glomerular function. Complete responses to NACT administration were achieved by 20% (2/10) of the patients, while partial responses were achieved by 30% (3/10) of the patients. The lesions of 30% (3/10) of patients stabilized, and 20% (2/10) of patients had no observed sensitive response to chemotherapy, and their lesions progressed after NACT. These final two patients included a patient diagnosed with small-cell carcinoma and a patient with stage IIB cancer.

Post-delivery treatment occurred in 65.8% (25/38) of patients (the PDT sub-group). Among them, eight had stage IB1, eight had stage II, two had stage IA, three had stage IB2, and four had stage IIIb cancer. Twenty-three of the 25 (92%) patients were diagnosed in the third trimester, at an average GA of 37 weeks (9–41 weeks) weeks, while the other two patients were diagnosed at 26 weeks and nine weeks of gestation, respectively.

Of the 38 patients in the COP group, a cesarean section was performed on 97.4% (37/38), and only one had a vaginal birth. Twenty-nine (76.3%) of the patients had premature births, while the other 9 (23.7%) had term deliveries with a mean GA of 35.0 $\pm$ 3.2 weeks. There were no significant differences among the three patient management subgroups (i.e., surgery, NATC, and PDT sub-groups) in GA at delivery (**Table 2**).

The treatments that patients in the COP groups underwent included:

- cesarean delivery combined with radical hysterectomy in three patients,
- post-delivery radical hysterectomy plus chemotherapy in 11 patients,
- post-delivery radical hysterectomy plus concurrent chemoradiotherapy (CCRT) in eight patients,
- post-delivery hysterectomy in three patients,
- post-delivery CCRT in four patients, and
- no treatment or unclear treatment in nine patients.

The median GA of the 67 patients in the TOP group at delivery was 15.2 $\pm$ 8.3 weeks. Among the 36 patients diagnosed in the first trimester, radical hysterectomy was performed on 18, abortion followed by radical hysterectomy on 13, abortion followed by CCRT on two, and details of the post-abortion treatment were not available for three patients. Among the 25 patients diagnosed in the second trimester, radical hysterectomy was performed on five, hysterotomy before radical hysterectomy on 13, and abortion followed by radical hysterectomy on seven patients. Radical hysterectomy following hysterotomy was performed on all six patients who were diagnosed in the third trimester. Post-operative adjuvant therapy was given according to the appearance of clinical high-risk factors.

Patient Outcomes

The median follow-up time for the 105 patients was 61 $\pm$ 6 months (1-173). There were no significant differences in follow-up time between the COP and TOP groups (40 vs. 45 months, $p = 0.532$). During follow-up, 11 patients (10.5%) experienced a relapse in cancer (six in the TOP and five in the COP groups), eight (7.6%, five in the TOP and three in the COP groups) died of tumor progression, and 21 patients (20%, six in the COP and 15 in the TOP groups) were lost to follow-up. None of the COP patients who were administered NACT showed any evidence of disease recurrence and death. The unadjusted hazard ratio between the COP and TOP groups was 1.063 for overall survival (95% CI = 0.24, 4.71). **Figure 1** displays the Kaplan–Meier curves for differences in survival since the diagnosis of cervical cancer between the COP and TOP groups ($p = 0.964$).

Fetal Outcomes

In terms of obstetric outcomes, data on fetal outcomes at birth were collected and analyzed from 38 patients with CCIP. The median weight of newborns from the COP group was 2,554 $\pm$ 760 g (1,315–4,050 g). Six of the 38 (15.8%) newborns were in the lower 10th percentile of weight as compared with the normal weight of newborns at the same GA. This is typically identified as small for gestational age. With the exception of four newborns for whom information was unavailable, 32 of the newborns had Apgar scores $\geq$7 at birth, with only two having an Apgar score below seven. Thirty-three children who completed follow-up visits with full records, during an average of 18 $\pm$ 2.8 months (0.7–84 months) of follow-up, showed normal physical and cognitive development. Details of the 38 COP cases are shown in **Table 3**.

DISCUSSION

Epidemiology of Cervical Cancer in Pregnancy in China

Due to a lack of specific data from randomized trials on the prevalence of CCIP in China, our study retrospectively assessed available records and determined the incidence of CCIP in China to be 0.020%, similar to what has been reported previously (13). The 35 years old median age at CCIP diagnosis suggests that childbearing at an older age might account for increased CCIP occurrence. It is worth mentioning that more than 90% of the CCIP cases were at least stage IB1 at the time of diagnosis. Squamous cell carcinoma was the most common subtype of CCIP and, therefore, characterized the disease's clinical and epidemiological picture (4). Vaginal bleeding was the main complaint, which might often be mistaken for potential miscarriage when the patient visited the

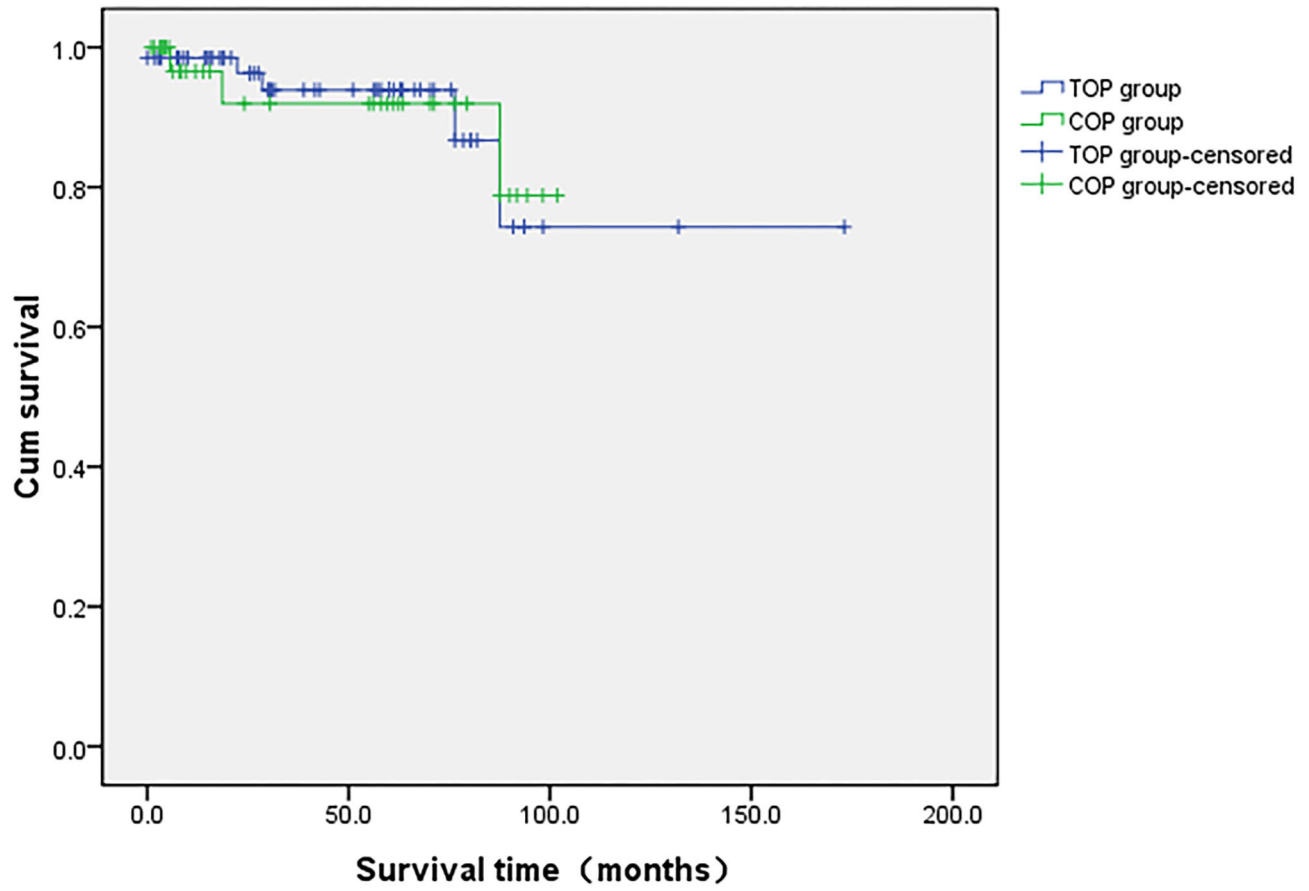

FIGURE 1 | Comparison of overall survival in the COP and TOP groups.

clinic in early pregnancy or be misdiagnosed as premature labor or placenta previa when the patient visited the clinic in mid or late pregnancy. Surprisingly, 72.4% of the patients with CCIP included in our study had not been screened for cervical cancer within the 5 years that preceded diagnosis. This indicates that the HPV vaccine and cervical cancer screening are not enough in monitoring and preventing this type of cancer in China (14). Given this, the Chinese Society for Colposcopy and Cervical Pathology (CSCCP) has recommended cervical cancer prevention via regular screening and early detection should be integrated into public education about women's health (15).

Management of CCIP Patients in China

There is not enough evidence to indicate the optimal treatment for CCIP patients. However, guidelines for the optional treatment of cervical cancer in pregnant patients have recently been published, which include expert recommendations based on limited data on cancer treatment efficacy during pregnancy (6, 7). Treatment decisions must consider many factors, including GA at diagnosis and patients' preferences for pregnancy outcomes. When the continuation of the pregnancy is not the purpose, management is similar to non-pregnant women. In our study, 91% (61/67) of the TOP patients terminated their pregnancies during the first or second gestation trimesters, and 9% (6/67) were in the third trimester when the potential exists to continue a pregnancy by taking postponed treatment. This indicates that knowledge of active management using surgery or NACT should be strengthened for options aimed at the continuation of pregnancy.

When a patient with CCIP chooses to preserve the pregnancy, several surgery protocols exist for the early stages of the disease (i.e., stage IA1–IB2), such as large conization or simple trachelectomy, which is gaining support (16, 17). Radical trachelectomy was a recommended option for young patients with early invasive uterine cervical cancer who decided to preserve their fertility. Some cases of radical trachelectomy have revealed that this procedure was challenging for gynecologic oncologists and obstetricians because of its operative radicality and the extremely high risk of complications (e.g., fetal loss, excessive bleeding, and prolonged surgery). Thus, it is not commonly recommended (6). In our study, radical trachelectomy was performed in two patients whose gestational weeks of delivery were 36^{+3} and 34^{+2}, respectively. Of those two patients,

TABLE 3 | Perinatal and pregnancy outcomes of COP cases ($n = 38$).

No	Maternal age (years)	GA at diagnosis (weeks)	Stage	Pathology	GA at delivery (weeks)	Delivery mode	Delivery weight (grams)	Apgar score	Age at last follow-up
1	36	34^{+5}	IIIB	SC	35	CS	1,700	8–8–8	6Y
2	29	34^{+2}	IB1	SC	35	CS	2,380	9–9–10	6Y
3	35	27^{+4}	IB2	SC	31^{+5}	CS	1,350	9–9–10	3M
4	36	25^{+3}	IB1	SC	36^{+3}	CS	3,320	9–9–10	2M
5	45	41^{+1}	IIB	SC	41^{+2}	CS	3,400	9–9–10	ND
6	38	40	IB2	AA	40^{+2}	CS	ND	9–9–9	5Y
7	32	31	IIIB	SC	31^{+4}	VD	1,600	10–10–10	5Y
8	24	38	IIB	SC	38	CS	2,500	ND	3Y
9	23	26	IB1	AA	28	CS	ND	ND	2Y
10	28	37^{+1}	IIIB	SC	37^{+1}	CS	2,400	10–10–10	10M
11	38	41	IB2	SC	41	CS	4,050	9–9–10	8M
12	35	36	IIA2	SC	36	CS	3,000	9–9–9	6M
13	34	28^{+4}	IB1	SC	34	CS	1,315	9–8–8	4Y
14	27	20^{+3}	IB2	SC	35	CS	2,460	10–10–10	23M
15	23	22^{+5}	IIB	SC	35	CS	2,170	10–10–10	18M
16	34	32	IB2	SC	34	CS	ND	ND	1M
17	37	28^{+2}	IIB	AA	30^{+2}	CS	ND	ND	3M
18	32	26^{+5}	IVB	SCLC	32^{+5}	CS	2,500	9–9–9	7M
19	40	31^{+6}	IA1	SC	35	CS	2,980	9–9–9	7M
20	29	38^{+3}	IB1	SC	38	CS	3,850	10–10–10	7Y
21	40	37^{+2}	IIA2	SC	37^{+2}	CS	3,560	10–10–10	ND
22	27	35	IB1	SC	35	CS	2,060	9–10–10	ND
23	34	40	IIA1	SC	40	CS	3,320	10–10–10	ND
24	32	38^{+4}	IA1	AA	38^{+4}	CS	3,020	10–10–10	1M
25	29	34^{+6}	IIIB	SC	34^{+6}	CS	1,700	4–6–6	3M
26	25	18	IB1	AA	34^{+2}	CS	1,700	9–9–9	4Y
27	24	34^{+2}	IIB	SC	35^{+1}	CS	2,480	10–10–10	7Y
28	26	31^{+4}	IA1	SC	34^{+5}	CS	2,330	9–10–10	3Y
29	40	37^{+2}	IB1	SC	37^{+5}	CS	3,420	10–10–10	7Y
30	26	38^{+4}	IB1	SC	40	CS	3,020	10–10–10	2Y
31	34	9	IIB	SC	32^{+5}	CS	1,700	10–10–10	30D
32	26	32	IIa2	SCLC	32^{+4}	CS	1,750	6–8–8	ND
33	37	39^{+2}	Ib2	AA	39^{+2}	CS	3,000	10–10–10	2.5Y
34	32	19^{+3}	IIB	SC	32^{+3}	CS	1,900	8–10–10	20D
35	38	39^{+6}	Ib1	SC	39^{+6}	CS	3,600	10–10–10	4.5M
36	33	24^{+4}	Ib2	SC	35^{+3}	CS	2,610	9–10–10	8M
37	35	6	IA2	SC	38^{+3}	CS	3,290	10–10–10	1Y
38	35	26^{+6}	Ib1_	SC	34^{+2}	CS	2,375	9–10–10	6Y

SC, squamous cell carcinoma; AC, adenocarcinoma; SCLC, small cell carcinoma; CS, cesarean section; VD, vaginal delivery; ND, not determined; Y, year; Age: M, month; D, day.

one suffered from cancer recurrence, suggesting the importance of pelvic lymphadenectomy.

In patients with more advanced stages (i.e., IB2-IIA) of cancer, NACT is an alternative management option commonly administered during pregnancy (6, 10, 18). Proper application of NACT can stabilize the tumor, control the disease, prevent the tumor from dissemination, and postpone unanticipated delivery (19). There is growing evidence on maternal safety and satisfied obstetrical outcomes with NACT administration during pregnancy (9). A NACT regimen comprised of paclitaxel plus cisplatin may be a proper approach for patients with CCIP (20). In our study, two cases with larger volume tumors received NACT and showed good chemotherapeutic reactivity. However, two patients did not respond well to chemotherapy, of which one responded to CCRT and intra-arterial chemotherapy after delivery (21). This suggests that for patients who choose to

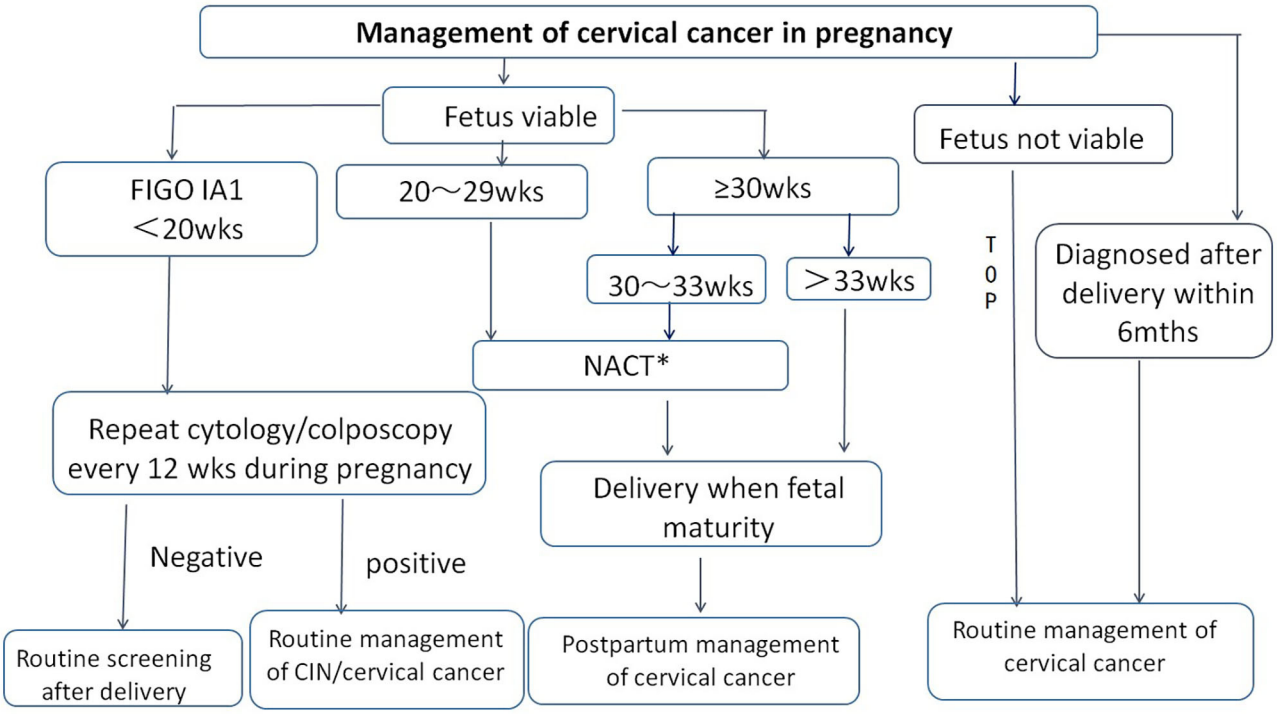

FIGURE 2 | Management of cervical cancer in pregnancy by CSCCP recommendation. TOP, termination of pregnancy; NACT, neoadjuvant chemotherapy; wks, weeks; mths, months. *For cervical cancer in pregnancy in stage IB1 and above, NACT may used as a treatment option to allow for fetal maturation during the second and third trimesters of pregnancy.

continue the pregnancy, postponing the treatment until the second trimester (when diagnosed during early pregnancy) or using NACT in the second and third trimester could be considered.

Maternal-Fetal Prognosis of Cervical Cancer in Pregnancy in China

Some literature has reported that CCIP has a poorer prognosis than cervical cancer in the general population of women due to its biological behavior. As a result, the proposed treatment protocols for CCIP were more active than those for more common cervical cancer (22). Increasingly, the literature has reported that pregnancy does not adversely affect the survival and prognosis of women with invasive cervical cancer (23–25). It is worth noting that our study did not find a difference in maternal survival between patients who terminated pregnancy and those who continued pregnancy. On the other hand, 33/38 children delivered to patients with CCIP were in good health and had no physical or intellectual disability at the end of follow-up. However, the effects of NACT administration on the fetuses of patients with CCIP and the development of long-term complications in children requires further monitoring and research (26).

Given the epidemic and treatment status of CCIP in China, consensus on the management of CCIP was achieved by the Chinese Society for Colposcopy and Cervical Pathology (CSCCP) in 2018 (**Figure 2**). This document may serve as a reference for the next prospective study and to promote the establishment of more standardized treatment guidelines for CCIP.

Limitations

Our study represents the largest Chinese trial of patients with CCIP, involving 17 hospitals from 12 provinces in northern, eastern, and central China. A weakness of our study is that 21 patients who had initially been included had no data on survival, which prevented us from obtaining an accurate estimate of maternal survival. Secondly, NACT was only applied in 10 cases in the COP group, which limited our power to demonstrate the efficacy and adverse effects of NACT. Furthermore, some infants were lost to follow-up, and the median follow-up was too short to provide mid- and long-term outcomes for infants. Adopting a retrospective design was inevitable to collect enough data for the outcome analyses. Consequently, this study design may have affected the descriptive statistics or survival analyses performed in the study cases.

CONCLUSION

In conclusion, educating the public about the importance of cervical cancer screening and the HPV vaccine is essential,

especially for those not being screened regularly before pregnancy. Administration of platinum-based NACT during the second and third trimesters is a safe and preferred option. When counseling patients on the treatment modalities available during pregnancy, it is important to consider that the oncologic outcome of patients who choose to continue a pregnancy is similar to those who choose to terminate the pregnancy.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/supplementary material.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics committee of Peking University people's hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

ML and LW conceived and designed the study. ML, YZhao, MQ, YZhan, LL, BL, RG, ZY, RA, JL, ZZ, HB, YH, SC, GH, KH, QZ, QL, YW, and JW collected and analyzed the data. ML and XL prepared the manuscript. LW and XL edited the manuscript. All authors contributed to data analysis, drafting and revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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REFERENCES

1. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, et al. Cancer statistics in China, 2015. *CA Cancer J Clin.* (2016) 66:115–32. doi: 10.3322/caac.21338
2. Di J, Rutherford S, Chu C. Review of the cervical cancer burden and population-based cervical cancer screening in China. *Asian Pac J Cancer Prev.* (2015) 16:7401–7. doi: 10.7314/APJCP.2015.16.17.7401
3. Wang B, He M, Chao A, Engelgau MM, Saraiya M, Wang L, et al. Cervical cancer screening among adult women in China, 2010. *Oncologist.* (2015) 20:627–34. doi: 10.1634/theoncologist.2014-0303
4. Hecking T, Abramian A, Domrose C, Engeln T, Thiesler T, Leutner C, et al. Individual management of cervical cancer in pregnancy. *Arch Gynecol Obstet.* (2016) 293:931–9. doi: 10.1007/s00404-015-3980-y
5. Pentheroudakis G, Pavlidis N. Cancer and pregnancy: poena magna, not anymore. *Eur J Cancer.* (2006) 42:126–40. doi: 10.1016/j.ejca.2005.10.014
6. Amant F, Berveiller P, Boere IA, Cardonick E, Fruscio R, Fumagalli M, et al. Gynecologic cancers in pregnancy: guidelines based on a third international consensus meeting. *Ann Oncol.* (2019) 30:1601–12. doi: 10.1093/annonc/mdz228
7. Amant F, Halaska MJ, Fumagalli M, Dahl Steffensen K, Lok C, Van Calsteren K, et al. Gynecologic cancers in pregnancy: guidelines of a second international consensus meeting. *Int J Gynecol Cancer.* (2014) 24:394–403. doi: 10.1097/IGC.0000000000000062
8. La Russa M, Jeyarajah AR. Invasive cervical cancer in pregnancy. Best Practice and research. *Clin Obstet Gynaecol.* (2016) 33:44–57. doi: 10.1016/j.bpobgyn.2015.10.002
9. Amant F, Han SN, Gziri MM, Vandenbroucke T, Verheecke M, Van Calsteren K. Management of cancer in pregnancy. Best practice and research. *Clin Obstet Gynaecol.* (2015) 29:741–53. doi: 10.1016/j.bpobgyn.2015.02.006
10. Perrone AM, Bovicelli A, D'Andrilli G, Borghese G, Giordano A, De Iaco P. Cervical cancer in pregnancy: analysis of the literature and innovative approaches. *J Cell Physiol.* (2019) 234:14975–90. doi: 10.1002/jcp.28340
11. Rob L, Skapa P, Robova H. Fertility-sparing surgery in patients with cervical cancer. *Lancet Oncol.* (2011) 12:192–200. doi: 10.1016/S1470-2045(10)70084-X
12. Pecorelli S. Revised FIGO staging for carcinoma of the vulva, cervix, and endometrium. *Int J Gynaecol Obstet.* (2009) 105:103–4. doi: 10.1016/j.ijgo.2009.02.012
13. Li M, Zhao Y, Guo R, Hua K, Lin B, L L, et al. Clinical analysis of 52 cases of cervical cancer during pregnancy. *Chin J Clin Obstet Gynecol.* (2018) 19:3–5. doi: 10.13390/j.issn.1672-1861.2018.01.0
14. Pan XF, Zhao ZM, Sun J, Chen F, Wen QL, Liu K, et al. Acceptability and correlates of primary and secondary prevention of cervical cancer among medical students in southwest China: implications for cancer education. *PLoS One.* (2014) 9:e110353. doi: 10.1371/journal.pone.0110353
15. Wei L, Zhao Y, Xie X, You Z, Bi H, Kong B, et al. Expert consensus management of cervical cancer during pregnancy in China. *Chin J Clin Obstet Gynecol.* (2018) 19:190–2. doi: 10.13390/j.issn.1672-1861.20
16. Covens A, Rosen B, Murphy J, Laframboise S, DePetrillo AD, Lickrish G, et al. How important is removal of the parametrium at surgery for carcinoma of the cervix? *Gynecol Oncol.* (2002) 84:145–9. doi: 10.1006/gyno.2001.6493
17. Strnad P, Robova H, Skapa P, Pluta M, Hrehorcak M, Halaska M, et al. A prospective study of sentinel lymph node status and parametrial involvement in patients with small tumour volume cervical cancer. *Gynecol Oncol.* (2008) 109:280–4. doi: 10.1016/j.ygyno.2008.02.004
18. Ilancheran A. Neoadjuvant chemotherapy in cervical cancer in pregnancy. Best practice andresearch. *Clin Obstet Gynaecol.* (2016) 33:102–7. doi: 10.1016/j.bpobgyn.2015.10.008
19. Rydzewska L, Tierney J, Vale CL, Symonds PR. Neoadjuvant chemotherapy plus surgery versus surgery for cervical cancer. *Cochrane Database Syst Rev.* (2012) 12:CD7406. doi: 10.1002/14651858.CD007406.pub3
20. Zagouri F, Sergentanis TN, Chrysikos D, Bartsch R. Platinum derivatives during pregnancy in cervical cancer: a systematic review and meta-analysis. *Obstet Gynecol.* (2013) 121(2 Pt 1):337–43. doi: 10.1097/AOG.0b013e31827c5822
21. Li M, Zhao Y, Liou Y, Zhu X, Wang J, Wei L. Neoadjuvant chemotherapy for locally invasive cervical cancer in pregnancy: two case reports. *Transl Cancer Res.* (2019) 8:1641–6. doi: 10.21037/tcr.2019.07.37
22. Xia T, Gao Y, Wu B, Yang Y. Clinical analysis of twenty cases of cervical cancer associated with pregnancy. *J Cancer Res Clin Oncol.* (2015) 141:1633–7. doi: 10.1007/s00432-014-1886-x

23. Zemlickis D, Lishner M, Degendorfer P, Panzarella T, Sutcliffe SB, Koren G. Maternal and fetal outcome after invasive cervical cancer in pregnancy. *J Clin Oncol.* (1991) 9:1956–61. doi: 10.1200/JCO.1991.9.11.1956
24. Germann N, Haie-Meder C, Morice P, Lhomme C, Duvillard P, Hacene K, et al. Management and clinical outcomes of pregnant patients with invasive cervical cancer. *Ann Oncol.* (2005) 16:397–402. doi: 10.1093/annonc/mdi084
25. Halaska MJ, Uzan C, Han SN, Fruscio R, Dahl Steffensen K, Van Calster B, et al. Characteristics of patients with cervical cancer during pregnancy: a multicenter matched cohort study. An initiative from the International Network on Cancer, Infertility and Pregnancy. *Int J Gynecol Cancer.* (2019) 29:676–82. doi: 10.1136/ijgc-2018-000103
26. Song Y, Liu Y, Lin M, Sheng B, Zhu X. Efficacy of neoadjuvant platinum-based chemotherapy during the second and third trimester of pregnancy in women with cervical cancer: an updated systematic review and meta-analysis. *Drug Des Devel Ther.* (2019) 13:79–102. doi: 10.2147/DDDT.S186966

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REVIEW
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Functional Alterations and Cerebral Variations in Humans Exposed to Early Life Stress

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Early life stress can be caused by acute or chronic exposure to childhood events, such as emotional, physical, sexual abuse, and neglect. Early stress is associated with subsequent alterations in physical and mental health, which can extend into adolescence, adulthood, and even old age. The effects of early stress exposure include alterations in cognitive, neuropsychological, and behavioral functions, and can even lead to the development of psychiatric disorders and changes in brain anatomy. The present manuscript provides a review of the main findings on these effects reported in the scientific literature in recent decades. Early life stress is associated with the presence of psychiatric disorders, mainly mood disorders such as depression and risk of suicide, as well as with the presence of post-traumatic stress disorder. At the neuropsychological level, the involvement of different mental processes such as executive functions, abstract reasoning, certain memory modalities, and poor school-skill performance has been reported. In addition, we identified reports of alterations of different subdomains of each of these processes. Regarding neuroanatomical effects, the involvement of cortical regions, subcortical nuclei, and the subcortical white matter has been documented. Among the telencephalic regions most affected and studied are the prefrontal cortex, the hippocampus, the amygdala, and the anterior cingulate cortex. Understanding the impact of early life stress on postnatal brain development is very important for the orientation of therapeutic intervention programs and could help in the formulation and implementation of preventive measures as well as in the reorientation of research targets.

Keywords: early life stress, prefrontal cortex, hippocampus, amygdala, post-traumatic stress disorder, substance use disorders, major depressive disorders, suicidal behavior

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HIGHLIGHTS

- The main neuropsychological alterations due to early exposure to stress occur in memory and executive functions.
- The main mental disorders related to early exposure to stress are substance use disorders, depressive disorders, and suicidal behavior.
- The hippocampus, the amygdala, and the prefrontal cortex are the brain structures in the human that have more alterations in relation to early life stress.

- All types of adverse childhood experiences increase the risk of psychoactive substance abuse.
- Very few studies address the differential cerebral and behavioral effect of early stress types in different socioeconomic conditions.

INTRODUCTION

Traumatic events in childhood can occur in different ways and under different characteristics and circumstances (1–3). Although there is no convergent and unambiguous classification of these events, they are currently grouped into two modalities: abuse (physical, sexual, emotional, or psychological) and negligence (2–4). Abuse and neglect are globalized phenomena that constitute a worldwide problem (5–8).

It has been suggested that adverse childhood experiences are associated with subsequent alterations in physical and mental health (9, 10). For instance, it has been reported that the greater the exposure of the infant to adverse situations, the greater the risk of suffering from pathologies such as cardiac ischemia, cancer, lung or liver disease, among others, in adulthood (9). Additionally, a greater predisposition for substance abuse, including alcohol and tobacco (11), has been described. Adverse childhood experiences have also been linked to unwanted pregnancies and the spread of sexually transmitted diseases (12, 13). Abuse in childhood increases the risk of suffering from a depressive episode at any point in life and has also been associated with lability in the response to treatment, as well as with a lack of remission during treatment for major depressive disorder (MDD) (14).

The effects of early stress have also been explored on a cognitive level. An important impact on the performance of tasks that require executive functions by subjects abused during childhood has been documented (15). Likewise, a strong association has been established between abuse and failures in different memory modalities, as well as poor development of school skills, and low scores on attentional tests and abstract reasoning (16, 17).

Neuroimaging methods have allowed the identification of correlations between neuroanatomic and functional alterations and early exposure to different types of traumatic events. In this regard, changes at the level of both cortical and subcortical structures have been documented (18, 19). Some reports have focused on the analysis of gross macroscopic changes, such as loss of cerebral parenchyma or increased ventricular space, while others have focused on specific structures such as the prefrontal cortex (PFC), hippocampus, amygdala, or cerebellum (10, 20–22). It is important to consider that despite the impact on the physical and mental health of those who have suffered from traumatic events in childhood, there are studies that suggest the possibility of different types of therapeutic interventions reversing or diminishing these effects (23–27).

The main mechanism underlying the deleterious effects of early stress is directly linked to the hypothalamic–pituitary–adrenal (HPA) axis. The HPA axis is related to the maintenance of homeostasis in the organism (28–31). Faced with situations that generate stress, this axis allows the suppression of the immune response, the mobilization of energy, and, in general, the activation of the sympathetic division of the autonomic nervous system. However, overexposure to stress and the consequent hyperactivity of the HPA axis during early stages of development have long-term effects on behavior and cognition, as well as on the brain structures that support the latter (10, 20–22). In a physiological context, the activation of the HPA axis is essential for the generation of a cascade of events that enable the organism to adapt. This cascade involves the paraventricular nucleus of the hypothalamus, which secretes arginine/vasopressin and corticotropin-releasing hormone (CRH), with the subsequent activation of the adenohypophysis and the release into the bloodstream of the adrenocorticotropic hormone (ACTH). ACTH acts peripherally on the adrenal cortex to produce glucocorticoids, which at the same time close a cycle of negative feedback on the hypothalamus for the inhibition of CRH and ACTH (32). It is important to consider that CRH exerts its action directly onto CRH receptors 1 and 2 (CRHR1/2), which are expressed not only in the adenohypophysis but also in the medial PFC (mPFC), the amygdala, the hippocampus, and the paraventricular nucleus. The predominant participation of CRHR1 in the onset of stress responses has been described, while CRHR2 appears to be more linked to the completion of responses (31–33).

The hyperactivity of the HPA axis can, in the first instance, alter the basal levels of glucocorticoids, cortisol in the case of humans. After prolonged exposure, it could affect the structural and functional organization of limbic structures such as the orbital PFC and mPFC, the cingulate gyrus, the amygdala, the hippocampus, and the hypothalamus, among others (31). These structural alterations are correlated with important changes at the functional level. For example, it has been shown that hypercortisolemia generates an important effect on the organization and function of the hippocampus, which has been evidenced in specific pathologies that manifest as an impairment of mental processes such as the short-term storage of information (34, 35). Volumetric analyses have found changes in subregions of the hippocampus and amygdala in subjects chronically subjected to different types of stimuli, such as physical and sexual abuse, during childhood. Likewise, alterations related to plasticity and neurogenesis (36, 37) have been found. Anatomo-functional variations secondary to the impact of the mechanisms involved in early stress have been documented, mainly in the PFC, predominantly in the medial and orbital surfaces, as well as

Abbreviations: PFC, Prefrontal cortex; mPFC, Medial prefrontal cortex; HPA, Hypothalamic–pituitary–adrenal axis; ACTH, Adrenocorticotropic hormone; CRH, Corticotropin-releasing hormone; PTSD, Post-traumatic stress disorder; BPD, Borderline personality disorder; MDD, Major depressive disorder; DD, Depressive disorder; ACC, Anterior cingulate cortex; ADHD, Attention deficit hyperactivity disorder; IQ, Intelligence quotient; NPMI, Non-psychotic mental illness; PDD, Pervasive developmental disorders.

in the amygdala and hippocampus, among other structures (10, 20–22).

This manuscript reviews the main findings reported over the past three decades on cognitive, behavioral, and psychiatric alterations, as well as the most notable brain structural changes associated with early stress in humans, regardless of the modality of the childhood traumatic event (be it abuse or neglect). The main scientific literature databases were used to search for bibliographic material: Redalyc, EBSCO, Web of Science, Scielo, PubMed, Scopus, Medline, and Google Scholar. The search terms were divided into two groups: the first group included terms that alluded to early stress or types of stressors, and the second group included terms that alluded to the types of structural and mental disorders of interest. Combinations of the two groups of terms were always used in the searches. Group 1: "early life stress," "adverse childhood experiences," "chronic stress," "childhood abuse," "childhood trauma," "sexual abuse," "physical abuse," "psychological abuse," "domestic violence," and "early neglect." Group 2: "cognition," "behavior," "executive functions," "neurobiological mechanism," "neuropsychological effect," "cognitive impairment," "psychiatric disorder," "cerebral effect," and "brain." A broad search parameter was established that considered relevant publications made since the last decade of the twentieth century. We privileged original publications in English, except for studies that reported unpublished data on the Latin American population in Spanish. Research carried out with biomodels was excluded, as well as research whose objective was to refer to changes associated with stress suffered in adult life. After initial screening, the authors scanned the titles and performed a second filter, which led to the analysis of the abstracts and, subsequently, to the full review of the articles. At the end of this process, 125 articles were included: eight published in the 1990's, 46 in the first decade of the twenty-first century, and the remaining 71 published in the past decade. Of these, five were published in Spanish and 120 were published in English.

The purpose of this manuscript was to identify the brain structures most affected by exposure to stress during childhood, as well as the cognitive, behavioral, and psychiatric functions presenting major alterations derived or associated with such exposure. In this way, not only is the information around this important issue for human health consolidated, but it also becomes visible and calls attention to the impact of early exposure to traumatic or stressful events on brain development, and therefore on mental health. This review will allow therapists to understand some of the symptoms and behaviors in neuropsychiatric pathologies arising from early exposure to stress and redirect their therapeutic efforts for the benefit of their patients' health. Likewise, this review may be useful for groups that are dedicated to research on the subject, because they may identify new questions or problems that can be addressed through scientific research, and for government organizations that monitor and ensure the healthy development of children and that fight for the health recovery of those who suffer the consequences of a traumatic childhood.

COGNITIVE, NEUROPSYCHOLOGICAL, BEHAVIORAL, AND PSYCHIATRIC ALTERATIONS IN HUMANS EXPOSED TO EARLY STRESS

Cognitive and Neuropsychological Alterations

The association between early chronic stress and changes in performance in cognitive and neuropsychological tasks has only been reported since the late 1990's (38–40). Among the studies collected in this review, it was possible to identify that cognitive and neuropsychological disturbances due to childhood stress can be organized into three groups: alterations in intelligence quotient (IQ), alterations in academic/school performance, and alterations in cognitive/executive functions (see **Table 1**).

Concerning the first group of alterations, few studies have reported the IQ of people who suffered childhood maltreatment. Some of these used the IQ variable to control the results of more complex and specific functions, such as executive functions, whereas other studies focused on the effect on intelligence of the relationship between childhood abuse and the later development of post-traumatic stress disorder (PTSD) (15, 41). For example, a study compared the performances on an intelligence scale in patients with PTSD and a history of abuse vs. subjects with a history of abuse without a diagnosis of PTSD, finding significantly lower scores on verbal tests exclusively in the population with a diagnosis of PTSD. Considering these findings, the authors suggested that it is PTSD and not childhood trauma that could be associated with verbal test deterioration (41). All the studies that looked for the relationship between early-life exposure to stress and changes in the general level of intelligence coincided in finding a deleterious effect of the former on the latter (see **Table 1**). The types of stress suffered by infants who later presented a low IQ level included neglect (44, 45) and institutional deprivation (47), witnessing domestic violence (42, 43), physical (41–43, 46), sexual (41, 46), or emotional abuse (46), and shooting, traffic accidents, smoke inhalation, and dog attacks (41). Specifically, one of these studies established that children who had witnessed violence in their homes exhibited a decrease on verbal component tests but not on manipulative and visuospatial intelligence tests, and in other studies, this same alteration in verbal IQ was presented in relation to other specific types of stress (41–43).

In the short time since investigative approaches to neuropsychological alterations associated with early life stress have been undertaken, it has been possible to identify that one of the clearest ways to obtain evidence of these alterations is through the analysis of academic achievements and school failures (38). However, the studies that emphasized these alterations were the fewest that were compiled in our review (38, 48), and generally, those that considered them did so in relation to PTSD (45, 46). All the research on this matter coincides in assessing the performance of people with a history of early child abuse in the field of mathematics (see **Table 1**). One study on the relationship between child abuse or neglect

TABLE 1 | Cognitive and neuropsychological alterations associated with exposure to early life stress.

Finding	Type of stress suffered	References
IQ		
Lower IQ	Abuse	(15)
Lower verbal IQ	PA or SA, domestic violence, shot, traffic accidents, smoke inhalation, dog attack	(41–43)
Lower IQ	Neg	(44, 45)
Lower IQ	Unspecified maltreatment (including PA, EA, and/or SA, domestic violence)	(46)
Lower IQ	Early institutional deprivation	(47)
Scholastic and/or academic failure		
Grade repetitions and lower performance in math and English	PA, SA, or Neg	(38)
LP in reading and math	Neg	(45)
LP in reading and math	Unspecified maltreatment (including PA, EA, and/or SA, domestic violence)	(46)
LP in math	SA (only women)	(48)
Cognitive/executive functions		
LP in recognition memory	PN and/or EA	(49)
LP in verbal memory	Abuse and/or domestic violence	(50)
LP in short-term verbal memory	PA and/or SA	(39)
LP in verbal declarative memory	SA (only women)	(51)
No change in explicit memory (verbal and visual)	SA (only women)	(40)
LP in explicit memory (verbal and visual)	PA and/or domestic violence	(52)
LP in associative learning	PA	(53)
LP in instrumental learning and EF (CF)	PA	(54)
LP in verbal memory, EF (visual attention, PL and PS)	Neg	(45)
LP in language, visuospatial memory, and EF	Unspecified maltreatment (including PA, EA, and/or SA, domestic violence)	(46)
LP in short-term memory and EF	SA (only women)	(48)
LP in visual-motor integration and EF (AA, PS, ABS, and reasoning) HL in EF (PS, ABS, and reasoning)	Neg with PA Neg without PA	(55)
LP in EF (attention and ABS)	Abuse	(15)
LP in EF (WM and others)	PA and/or EA or Neg	(56, 57)
LP in EF (IC)	SA	(58)
LP in EF (attention, IC, and PL)	Neg	(59)
LP in EF (sustained attention) HV to acute stress on sustained attention	Experimental acute stress Parenting stress	(60)
LP in EF	Neg	(44)
LP in EF (WM, IC, AA, and processing speed)	Familial trauma	(61)
LP in EF (CF, WM, and IC)	Early institutional deprivation	(47)
LP in EF (targeted attention)	Early institutional deprivation	(62)
LP in EF (decision making and increased risk taking)	Early institutional deprivation	(63)
LP in EF (WM)	Household dysfunction	(64)

List of studies whose participants presented cognitive and neuropsychological alterations associated with exposure to early life stress, as well as the types of stress identified to which they were subjected.

IQ, Intelligence quotient; LP, Lower performance; HP, Higher performance; HV, Higher vulnerability; EF, Executive functions; PA, Physical abuse; EA, Emotional abuse; SA, Sexual abuse; PN, Physical neglect; Neg, Neglect; Household dysfunction, including death or severe chronic illness of family member or friend, severe marital conflict; PL, Planning; PS, Problem-solving; ABS, Abstraction; WM, Working memory; IC, Inhibitory control; CF, Cognitive flexibility.

and academic difficulties assessed a group of 300 students, based on three variables: decrease in school grades, number of grade repetitions, and performance in English and mathematics. The authors of this study found that abused children had a greater risk of incurring a decrease in grades and also a greater possibility of poor performance in either of the two subjects mentioned (38). The other three studies that addressed the relationship between early stress and school performance assessed reading

and mathematics in sexually abused girls (48) or in men and women who suffered from neglect (45) or various types of unspecified abuse (46) and all agreed in finding a significant impairment in these issues.

Research addressing variations in performance in cognitive/executive tasks due to early exposure to stress was the most abundantly found in this section of our literature search. The evaluated functions included language, visual-motor integration, instrumental and associative learning, various types of memory, visual and auditory attention, as well as the executive functions themselves (see **Table 1**). A significant number of the studies evaluated the involvement of some type of specific memory, other studies combined the evaluation of executive functions with one or more of the functions mentioned above, and the vast majority exclusively evaluated one or more of the executive functions. Only one investigation considered recognition memory, finding low performance in tasks that required it in children who experienced neglect and/or emotional abuse and who also presented hypocortisolism (49).

Several studies have focused on the assessment of verbal memory in individuals who suffered different types of abuse in their childhood; all except one found poor performance in the tasks presented for this purpose. Specifically, one of the studies was done in people exposed early to abuse and/or domestic violence (50); another study found short-term verbal memory impairment in people subjected to physical and/or sexual abuse (39); two investigations focused exclusively on sexually abused women, one found alterations in verbal declarative memory (51) and the other found no changes in explicit verbal and visual memories (40). This last study is opposed by the findings of another in which explicit verbal and visual memories were found to be impaired in individuals who had suffered physical abuse and domestic violence (52). Finally, one more investigation found that verbal memory as well as some executive functions were affected in neglected people (45). Additionally, in a study that also evaluated other cognitive processes, visuospatial memory was found to be altered in individuals subjected to various types of maltreatment (46), poor performance in short-term memory tasks occurred in sexually abused women (48), and associative (53) and instrumental (54) learning were also altered in victims of childhood physical abuse.

Other higher mental functions have also been found to be altered in response to stress suffered during childhood, such as visual-motor integration in people who suffered neglect with physical abuse (55) and language in those who suffered any of the types of abuse or domestic violence (46).

Executive functions are the most studied higher mental functions and also those in which there are more alterations in response to early life stress (44, 46). These executive functions include planning, problem-solving, attention, abstraction, reasoning, working memory, inhibitory control, decision making, and cognitive flexibility (see **Table 1**). Thus, several studies have found that early institutional deprivation significantly affects functions such as working memory, cognitive flexibility, inhibitory control (47), targeted attention (62), and decision-making (63). Working memory has also been found to be altered in those who suffered early physical and/or emotional abuse or neglect (56, 57), family trauma (61), or some type of household dysfunction (64). Additionally, poor performance has been found in cognitive flexibility tasks in physically abused people (54), and in inhibitory control tasks in those who were sexually abused (58), who suffered neglect (59), or some family trauma (61).

Attention is another of the executive functions evaluated in individuals who were exposed to early stress (15, 59), in its visual (45), auditory (55, 61), and sustained (60) modalities. Attention was found to be impaired in all study participants, whether they had been abused (15), neglected (45, 59), exposed to the combination of physical abuse with neglect (55), or exposed to family trauma (61). An investigation found that children exposed to an acute stressful experimental event had their sustained attention altered, and the vulnerability of attention increased when they were exposed to parental stress (60).

Problem-solving (45) and planning (45, 59) have also been found to be impaired in people who suffered from early neglect. Paradoxically, one study found that problem-solving, abstraction, and reasoning were affected when participants had suffered neglect plus physical abuse, but their performance in these executive functions was better than that of non-maltreated people who had only suffered neglect (55).

In summary, 13 studies included physically abused individuals, in which alterations in the IQ and in school capacities as well as in cognitive and executive functions were evaluated; six studies included sexually abused people (predominantly women) and evaluated almost exclusively executive functions; and the same number of studies and type of functions evaluated people who suffered childhood neglect. Three investigations considered individuals who suffered early institutional deprivation, assessing their executive functions, and another six studies evaluated people with a childhood history of domestic violence, family trauma, or some type of household dysfunction. Studies that assessed alterations in cognitive/executive functions associated with early exposure to stress were more abundant than those that assessed alterations in the level of general intelligence or in school performance. There seemed to be a consensus among the studies that assessed the IQ of those who suffered some type of abuse in childhood, that these people had a lower level of general intelligence than those who were not abused. However, more studies are required on this issue that address not only IQ but also all cognitive and neuropsychological spheres that allow ratifying this finding and deepening it, being precise in identifying the type of stressor (or types of stressors) to which people were subjected.

Specifically, more studies should be done on school disorders and the general intelligence of people who suffer from any type of abuse in childhood, regardless of whether or not they have PTSD. Among those who present school failures, the relationship between exposure to early stress and performance in other fields of knowledge such as social sciences, natural sciences, and sports activity should also be evaluated.

Behavioral and Psychiatric Disorders

The suffering of adverse experiences in childhood has been associated with the subsequent emergence of multiple alterations

in physical (65–70) and mental health (71, 72). Although psychiatric and behavioral disorders include those that could compromise the lives of people exposed to traumatic events in their childhood, practically all investigations found were undertaken in this century, except for one in 1985 (73) and another in 1999 (74). In this section, we will address some of the most significant behavioral changes and psychopathological implications reported in the literature. The research collected in our review allows us to appreciate that psychiatric and behavioral disorders associated with early stress belong to one of three large groups: substance use disorders, depressive disorders, and suicidal behavior (see **Table 2**). It is important to remember that in the previous section, we pointed out that PTSD is the most common disorder associated with neuropsychological and cognitive alterations, and as can be seen in **Tables 3–5**, it is also a common disorder among people with cerebral anatomical and functional alterations after childhood maltreatment.

Nine studies found in this section of our review included people with some type of substance use or abuse disorder (at any point in life from puberty to older adulthood) who were exposed to early stress. Of these investigations, only one included exclusively women (74), one included exclusively cocaine abusers (81), three included exclusively alcohol abusers (78–80), and five included abusers of various types of substances (11, 74–77). In all these investigations, the types of stress suffered were physical and sexual abuse. Most of them also included participants who would have been exposed to emotional abuse (11, 74, 75, 78–81), and physical or emotional neglect (11, 74, 78, 79, 81). Additionally, two studies included participants who suffered neglect but the type was not specified (76, 77), and another two included people who were exposed to some type of household dysfunction (11, 80), which may have included battered mother, household substance abuse, death or mental illness in the household, parental separation or divorce, and criminal or incarcerated household member.

From these studies on the relationship between substance use disorders and early exposure to stress, some interesting contributions can be highlighted, for example: (A) By retrospectively examining the relationship between the use of illicit drugs and 10 categories of childhood adversity in a cohort of 8,613 subjects, it was found that all the categories increased between two and four times the probability of early consumption initiation, and that when comparing people with and without a history of childhood adversity, those who obtained a higher score on the adversity scale were between 7 and 10 times more likely to report the use of illicit substances and/or addiction to them (11). (B) In a study carried out with 587 participants, a direct correlation was found with the degree of physical, sexual, and emotional abuse in childhood. Cocaine was the substance most associated with the severity of the trauma (75). (C) When assessing the prevalence of childhood trauma in alcoholic patients with therapeutic treatment, it was evidenced that alcohol dependence was more frequent and severe with the presence of a traumatic history. Emotional abuse was the clearest predictor for alcohol consumption, followed by physical abuse (78). (D) Differences in consumption patterns have also been observed with respect to gender. In men, emotional abuse was associated with an earlier age for the initiation of alcohol consumption, as well as with an increase in its severity. In women, sexual and emotional abuses were the variables most strongly associated with the age of initiation of alcohol consumption (81).

TABLE 2 | Behavioral and psychiatric disorders associated with exposure to early life stress.

Disorder	Type of stress suffered	References
SUDs	PA, EA, SA, PN, EN, household dysfunction	(11)
SUDs	PA, EA, or SA	(75)
SUDs	PA and/or SA and/or Neg	(76, 77)
SUDs (only women)	PA, EA, and SA; PN and EN	(74)
SUDs (alcohol)	PA, EA, and SA; PN and EN	(78, 79)
SUDs (alcohol)	PA, EA, and SA; household dysfunction	(80)
SUDs (cocaine)	PA, EA, and SA; PN and EN	(81)
Alexithymia	PA, EA, and SA; Neg	(82)
MDD	PN and EN	(83)
MDD	PA, EA, and SA; Neg and domestic violence	(84)
Depressive disorders (MDD and dysthymia)	PA, EA, and SA, household dysfunction	(85)
Depressive symptoms	PA, EA, or SA	(86)
Depression	PA, EA, and SA; PN and EN	(87)
Suicidal ideation	PA, SA, and EA; neglect	(88)
Suicidal behavior	Abuse and neglect	(73)
Suicidal behavior	SA	(89, 90)
Suicidal behavior	PA and/or SA	(91)
Suicidal behavior (only women)	PA and/or SA	(92)
Suicidal behavior	PA, EA, and SA; household dysfunction	(93)
Suicidal behavior	PA, EA, and SA; Neg, household dysfunction	(94)
Suicidal behavior	PA, EA, and SA; PN and EN	(95)
Depressive symptoms and suicidal behavior	PA, EA, and SA; PN and EN	(96)

List of studies whose participants presented behavioral and psychiatric disorders associated with exposure to early life stress, as well as the types of stress identified to which they were subjected.
SUDs, Substances use disorders; MDD, Major depressive disorder; PA, Physical abuse; EA, Emotional abuse; SA, Sexual abuse; PN, Physical neglect; EN, Emotional neglect; Neg, Neglect; Household dysfunction, including battered mother, household substance abuse, death or mental illness in household, parental separation or divorce, criminal, or incarcerated household member.

In the second group of psychiatric disorders, we found six studies that included people with depressive symptoms or disorders who were exposed to childhood stress. Of these investigations, three were specifically in individuals diagnosed with MDD (83–85) and three with participants presenting depression or depressive symptoms (86, 87, 96). There was no specific type of early stressor common among all the studies done in people with a diagnosis of depressive disorder. However, the types of stressors suffered in childhood included physical, emotional, and sexual abuses in five of six investigations (84–87), physical and emotional neglect in four of six investigations

TABLE 3 | Variations in the prefrontal cortex associated with exposure to early life stress.

Cerebral finding	Cognitive, neuropsychological, behavioral, and psychiatric alterations	Type of stress suffered	References
Ventromedial PFC volume increase and dorsomedial PFC volume decrease	Impaired social performance, poor school performance, general distress, regressive behaviors, PTSD, DD, MDD, social phobia, ADHD, anxiety, and simple phobia	PA, EA, or SA, separation or loss, witness of violence, PN, multiple exposure	(97)
Bilateral increase in ventromedial PFC volume	PTSD, DD, MDD, social phobia, ADHD, anxiety, and simple phobia	PA, EA, or SA, separation or loss, witness of violence, PN, multiple exposure	(98)
Left SFG volume decrease	Anxiety and/or DD	EN and/or psychological abuse	(99)
Decrease in total gray matter volume of PFC	MDD	PN and EN	(83)
Decreased connectivity of the right dorsomedial (BA10) and left dorsomedial (BA9) PFC	Verbal IQ 10 points below the control group and attention commitment	Severe physical punishment	(100)
Medial PFC hypoactivity during coding and evocation with emotional worth	Anxiety and/or DD	Emotional stress	(101)
Dorsomedial PFC hyperactivity	NPMI, PDD, and/or SUDs, alexithymia	Chronic emotional abuse	(102)

List of studies that reported structural changes in the prefrontal cortex with associated behavioral, cognitive, and psychiatric alterations, as well as the types of early stress identified in participants.
PFC, Prefrontal cortex; SFG, Superior frontal gyrus; PTSD, Post-traumatic stress disorder; DD, Depressive disorder; MDD, Major depressive disorder; ADHD, Attention deficit hyperactivity disorder; IQ, Intelligence quotient; NPMI, Non-psychotic mental illness; PDD, Pervasive developmental disorders; SUDs, Substances use disorders; PA, Physical abuse; EA, Emotional abuse; SA, Sexual abuse; PN, Physical neglect; EN, Emotional neglect.

TABLE 4 | Variations in the hippocampus associated with exposure to early life stress.

Cerebral finding	Cognitive, neuropsychological, behavioral, and psychiatric alterations	Type of stress suffered	References
Global volume increase	Undetermined	PA, EA, or SA, Neg and domestic violence	(103)
Right hippocampus volume decrease	Correlation with severity of PTSD symptoms	PA, EA, or SA, Neg and separation	(104)
Left volume decrease	MDD	PA and/or SA (only women)	(105)
Left volume decrease in CA2, CA3, and CA4, Dentate Gyrus, subiculum, and presubiculum	Undetermined	PA and/or verbal abuse	(106)
Global volume decrease	MDD	PA, EA, or SA, PN, separation or loss, illness, life-threatening injury	(107)
Bilateral volume decrease and left hippocampus hypoactivity in verbal memory tasks	PTSD	SA (only women)	(108)
Bilateral volume decrease	Undetermined	SA (only women)	(109)
Bilateral volume decrease	Disruptive behavior	PA	(72)
Bilateral volume decrease	PTSD; verbal memory deficit	PA and/or SA	(110)
Bilateral volume decrease	BPD	PA, EA, or SA, Neg and separation	(111)
Bilateral volume decrease	BPD	PA and/or SA (only women)	(112)

List of studies that reported structural changes in the hippocampus with associated behavioral, cognitive, and psychiatric alterations, as well as the types of early stress identified in participants.
PTSD, Post-traumatic stress disorder; MDD, Major depressive disorder; BPD, Borderline personality disorder; PA, Physical abuse; EA, Emotional abuse; SA, Sexual abuse; PN, Physical neglect; EN, Emotional neglect; Neg, Neglect.

TABLE 5 | Variations in the amygdala associated with exposure to early life stress.

Cerebral finding	Cognitive, neuropsychological, behavioral, and psychiatric alterations	Type of stress suffered	References
Left volume decrease	Disruptive behavior	Abuse, neglect, and socioeconomic deprivation	(72)
Volume decrease and altered neurophysiological response of fear to conditioned stimuli	Anxiety, DD, PTSD, Externalizing Disorders	PA or SA, and domestic violence	(113)
Volume decrease	BPD	PA, EA, or SA, Neg and separation	(111)
Bilateral volume decrease	BPD	PA or SA (only women)	(112)
Global volume increase	No report	Early institutional deprivation	(114)
Volume increase	Dysregulation of emotions and anxiety	Early institutional deprivation	(115)
Bilateral volume increase	No report	Neg	(116)
Left volume increase	Undetermined	PA, EA, or SA, Neg, domestic violence	(103)
Right hyperactivity in face recognition paradigms with emotional worth	Behavioral problems and hyperactivity	PA, EA, or SA, Neg	(117)
Early maturation of functional connectivity between amygdala and mPFC	Anxiety	Early institutional deprivation	(118)
Functional hyperactivity and hyperconnectivity between amygdala and mPFC	Internalizing disorders	Household dysfunction	(119)
Functional connectivity increase between amygdala and mPFC	Undetermined	Insensitive parenting	(120)
Right hyperactivity of functional connectivity between amygdala and ACC	DD	SA	(19)
Functional connectivity decrease between amygdala and mPFC	Aggressive behaviors and attention problems	Household dysfunction	(121)

List of studies that reported structural changes in the amygdala with associated behavioral, cognitive, and psychiatric alterations, as well as the types of early stress identified in the participants.

PTSD, Post-traumatic stress disorder; DD, Depressive disorder; MDD, Major depressive disorder; BPD, Borderline personality disorder; mPFC, Medial prefrontal cortex; ACC, Anterior cingulate cortex; PA, Physical abuse; EA, Emotional abuse; SA, Sexual abuse; Neg, Neglect; Household dysfunction, including maternal depression, negative parenting, family anger, maternal role overload, financial stress, and death of family member or divorce.

(83, 84, 87, 96), and some type of family death or domestic violence in two of six investigations (84, 85). The most important findings reported in studies that addressed the relationship between early stress and the subsequent emergence of depressive disorders in adolescence and adulthood included the following: (A) Emotional abuse in childhood increases the lifetime risk of depression 2.7 times in women and 2.5 times in men (85). (B) Emotional abuse is the type of early stress most strongly related to depression and possibly related to the pathogenesis of the disease (87).

The greatest number of investigations on psychiatric and behavioral disorders in people who have suffered some type of child maltreatment have addressed the population that exhibits suicidal ideation or suicidal attempts (10 of 25 articles in our review). Only one study focused on people with suicidal ideation (88), the rest were on those who presented suicide attempts (73, 89–96). Sexual abuse appears to be the most common type of stressor experienced by participants with suicidal ideation or suicide attempts, as it was found to be associated in all the studies reviewed with this behavioral disorder. In descending order, physical abuse was reported in seven out of 10 studies (88, 91–96), emotional abuse was reported in five out of 10 studies (88, 93–96), some type of neglect was reported in five out of 10 studies (73, 88, 94–96), and some type of family dysfunction was reported in two out of 10 studies (93, 94). Only one investigation evaluated exclusively abused women with a history of suicide attempts (92), and another evaluated suicide risk exclusively in abused older adults early in life (96).

Among the most important findings reported in studies that addressed the relationship between early stress and the subsequent emergence of suicidal ideation or attempt are the following: (A) Regardless of the type of early adverse experience, the risk of suicide increases between two and five times in adolescents and adults (93), and among those who entered the department of social services during their childhood, the suicide risk was three to six times higher (73). (B) Child sexual abuse appears to be a direct predictor of later suicidal ideation (88), and sexually abused individuals had a suicide rate between 10.7 and

13 times higher than the average rate of the general non-suicidal population (89). (C) Sexual and physical abuses in childhood were correlated with recurrence of suicide attempts (94). (D) The identity of the abuser is related to the number and frequency of suicide attempts, and sexual abuse perpetrated by some of the members of the family itself generated a greater risk of suicide (91). (E) Among sexually abused men and women, the former are more likely to make multiple suicide attempts and to be diagnosed with borderline personality disorder (BPD) or PTSD (90).

In summary, there is growing evidence of the relationship between early stress and the subsequent emergence of psychiatric and behavioral disorders, mainly substance use disorders, depressive disorders, and suicidal behavior. Predominantly, the types of stressors related to these disorders are physical, sexual, and emotional abuse; therefore, public policies for the prevention of child abuse should focus on these.

STRUCTURAL ALTERATIONS IN THE HUMAN BRAIN EXPOSED TO EARLY STRESS

Prefrontal Cortex

The PFC, particularly its dorsolateral portion, is the region of greatest expansion and most recent phylogenetic acquisition of the human cerebral cortex. Its role is to orchestrate and regulate emotions, actions, and thoughts, through the establishment of an intricate network of connections, both with itself and with other cortical and subcortical structures. Functionally, it has been related to working memory, attention processes, impulse inhibition, cognitive flexibility, error monitoring, and planning, among others (122–126). The PFC is perhaps the most immature cortical region at birth and its maturation process ends late (between adolescence and early adulthood); thus, it presents a particular vulnerability to stress stimuli and adverse situations that occur chronically during childhood and adolescence. This has been documented in research with humans and with biomodels. In fact, a negative impact on its functioning has been reported even with acute exposure. In the face of prolonged exposure, both macroscopic structural changes and alterations at the level of the microscopic organization of neurons (10) have been evidenced.

In addition to the alterations in functional connectivity between the PFC and the amygdala mentioned in Section amygdala (19, 121, 127, 128), a similar number of investigations have evidenced structural changes and alterations in the level of activity of regions of the PFC associated with early exposure to stress (see **Table 3**). Such is the case of a study conducted in 23 children who had suffered early trauma and presented positive signs of PTSD, in which variants in the macroscopic organization of the PFC were analyzed. The results indicated that the gray matter of the mPFC of children with PTSD had a significantly greater volume in the ventromedial region and a volumetric decrease in the dorsomedial region, which was positively correlated with the functional impairment of children (97). Another study, also carried out in children diagnosed with PTSD, reported a greater volume of gray matter in the mPFC of both hemispheres, predominantly over the ventral region, which led them to suggest the existence of an association between PTSD and structural changes in the mPFC, as well as a possible correlation between this change and the severity of symptoms (98).

Physical abuse in childhood has been associated with alterations in behavior and mood, as well as with changes in PFC volume. A study found a 19.1% reduction in the cortical volume of the dorsomedial connectivity of the upper right frontal gyrus [corresponding to Brodmann area 10 (BA10)] and a 14.5% reduction in the dorsomedial connectivity of the upper left frontal gyrus (corresponding to BA9). The findings of this study established a positive correlation between cortical volume and the IQ of the subjects (100).

A study with patients diagnosed with depression and/or anxiety who had been exposed to emotional abuse in childhood showed a significant reduction in the dorsal portion of the medial surface of the left superior frontal gyrus. This decrease was independent of whether there was physical or sexual abuse, of the concomitant pathology, and of the sex of the participants (99). Moreover, in adult patients diagnosed with major depression and a history of early stress, a significant reduction in the overall volume of the gray matter of the PFC was observed, which was positively correlated with physical and emotional negligence (83).

An investigation based on functional magnetic resonance imaging (MRI) analyzed the differential activation of the mPFC during the coding and recognition of positive, negative, and neutral words in patients diagnosed with depression and/or anxiety who had suffered neglect, physical, emotional, or sexual abuse before 16 years of age. A hypoactivation of the mPFC was found during the coding and evocation of emotionally valuable stimuli in subjects exposed to emotional stress during childhood. This hypoactivation could not be explained by variables such as the type of psychopathology, the severity of the symptoms, the use of medication, or the decrease in the volume of the cortex (101). The same authors warned about the functional susceptibility of the PFC in response to exposure to a social exclusion paradigm, which was linked to increased activity of the ventromedial PFC and hyperactivity of the dorsomedial PFC, depending on the severity of the abuse (102).

Hippocampus

The hippocampus is one of the brain structures directly related to memory and learning (129) on which changes associated with exposure to stress in the early stages of development have been reported with greater consistency (see **Table 4**) (110, 113). Since the last century, after the advent of diagnostic imaging techniques, it was possible to notice macroscopic changes in the hippocampus of individuals exposed to sexual abuse during childhood. When comparing a sample of 17 adult abuse survivors with 17 non-abused subjects, matched by race, age, sex, body size, and years of education, among other factors, a 5% reduction in the total volume of the right hippocampus and a 12% reduction in the left hippocampus were found, the latter representing a significant difference. No differences were found in other brain structures such as the amygdala, the

caudate nucleus, or the entire temporal lobe (110). A functional asymmetry has been documented concerning the difference in hippocampal size reduction, linking the left hippocampus predominantly with short-term verbal memory, vs. the right one, predominantly associated with visual memory (39, 129). Correspondingly, specific short-term verbal memory deficits have been reported in subjects who have suffered sexual abuse during childhood (39, 130). Similar structural findings were reported when analyzing volumetric changes in the hippocampus of adult (non-medicated) right-handed subjects who suffered early abuse, which showed a strong association between abuse and decreased volume of Ammon's Horn 2 (CA2), CA3, and CA4, as well as the dentate gyrus, both of the left hippocampus. The CA2 and CA3 reduction was 6.3%, and the CA4 and dentate gyrus reduction was 6.1%. The presubicle and subicle of the same hemisphere also exhibited a reduction of 4.2 and 4.3%, respectively. Other structures such as CA1 and fimbria presented no significant reductions (106).

There have also been documented changes at the hippocampus level in patients with different types of psychopathology and in patients who have suffered early stress (103, 107). In a pilot study whose participants were subjects between 7 and 13 years old and who suffered traumatic events during childhood (physical and emotional violence, separation, sexual abuse, or neglect), and who were diagnosed with PTSD, it was determined that the severity of symptoms and cortisol levels correlated negatively with the volume of the right hippocampus. The data had no statistical significance for the left hippocampus (104). Although most of the investigations have documented some type of change in the hippocampus of individuals diagnosed with PTSD who have been exposed to stress due to childhood trauma, there are a few in which no volumetric decrease has been evidenced (131); there was even one reported hippocampal widening when compared with controls (132).

A study based on MRI and positron emission tomography (PET) was performed in women with a diagnosis of PTSD who had or had not been sexually abused during childhood and in women without this antecedent and without any diagnosis. The results indicated that women with PTSD and childhood sexual abuse had a smaller hippocampus than women with PTSD diagnosis but no history of abuse. This same trend was observed when compared with controls. The average volume of the hippocampus was 16 and 19% lower, respectively. When specifying by hemisphere, the data corresponded to 15 and 17% for the left hippocampus and 16 and 22% for the right hippocampus. There was a hypoactivity of the left hippocampus in tasks of verbal memory in the PET of women with PTSD who had suffered sexual abuse in childhood (108). Although most of the results of research conducted with women with PTSD and childhood trauma pointed to the existence of a correlation with structural and functional changes in the hippocampus, it is worth mentioning that there are studies in which there were no differences (133).

On the other hand, in women with BPD and a history of childhood trauma, a bilateral reduction of the hippocampus volume close to 16% was found (111). Similar findings were subsequently also reported in a group of women with BPD and with high scores in the Early Trauma Inventory, which correlated with a history of physical and/or early sexual abuse. In this case, the reduction of the right hippocampus was around 16%, while the left hippocampus showed a reduction close to 10% (112).

In the case of women diagnosed with MDD, a study analyzed the structural changes in the hippocampus associated with physical and/or sexual abuse during pre-puberty. Out of the 32 women who participated, 21 had a history of abuse in childhood. On average, this group showed an 18% decrease in the left hippocampus when compared to depressed patients with no history of abuse. This difference was 15% compared with the control group. This study showed no changes in the volume of the right hippocampus among the three groups of women (105).

A differential analysis between the association of the volume of different brain structures and the specific modality of stress stimulation (physical abuse, neglect, and low socioeconomic status) during early life found that a bilateral decrease in the volume of the hippocampus was predominantly linked to subjects who had suffered physical abuse, and whose condition was due to a low socioeconomic level. This same study observed a directly proportional relationship between the level of accumulated vital stress, behavioral problems, and hippocampal volumetric loss (72).

Based on the evidence of the impact of early stress on the structure and function of the hippocampus, a hypothesis of the existence of critical periods in the development of this structure has been raised. A study conducted with a sample of 178 children between 9 and 13 years old, who were interviewed and asked about the severity and age of exposure to the stress stimulus, found a negative association between the severity of stress and the bilateral size of the hippocampus. This association, although small, was statistically significant, even without considering age. When the age of onset was considered, a moderate negative association was found between the severity of stress during early childhood (up to 5 years old) and the volume of the hippocampus. There was no correlation between the severity of stress and late childhood (above 6 years old) (134). Similar findings were shown in a study done with 26 women with a history of repeated episodes of childhood sexual abuse, evidencing the presence of two critical periods of hippocampal vulnerability for this specific type of stress stimulus. These periods were between 3 and 5 years old and between 11 and 13 years old (109).

These investigations allude to the impact that the environment has on neurotypical development, as well as to the existence of sensitive periods. In the case of the size of the hippocampus and the psychopathologies associated with its structural alterations, it has been revealed that parental care is the aspect that has the greatest implications, even above the effect of environmental stimulation. It is known that parenting children up to 4 years of age predicts the volume of the left hippocampus in adolescence. This association between parental care and volumetric hippocampal development only seems to exist until the person is 8 years old (135).

Amygdala

The amygdala has been linked to diverse functions, for example, to the recognition of faces with emotional value, to the control of behavior directed to an objective, to the evaluation of sensory information, which is important in adaptive terms, to memory, and to the evocation of emotionally relevant events, among others (136–139). Like the hippocampus, the amygdala seems to be particularly vulnerable to stressful events that occur early in life (see **Table 5**). In a comparative study, the volumetric changes of the amygdala were analyzed in relation to early stress, distinguishing between the types of stressor stimulus: abuse, neglect, or socioeconomic deprivation. The results of this study showed that there was a direct association between exposure to any type of abuse and a reduction in the size of the amygdalin nucleus. Similarly, there was a correlation between the cumulative stress load over time, behavioral disorders, and volume loss. In all cases, this phenomenon occurred exclusively for the left amygdala (72). Similar findings were presented when analyzing the physiological and behavioral responses to a fear learning and extinction paradigm in subjects exposed to physical, sexual, or domestic violence during childhood. This study showed deregulation of the mechanisms involved, as well as a generalized reduction in the volume of the amygdala (113).

In the United Kingdom, a pilot study was conducted with Romanian children who had been adopted and had suffered severe deprivation in childhood, after living in dire conditions during orphanhood. The objective was to quantify the effects of early stress on brain structure. The study found that subjects exposed to early stress had a reduction in the overall volume of the gray and white matter of the brain. However, after adjusting for total volume, there was a striking increase of ∼35.5% in the size of the amygdala when compared with healthy controls. This difference was observed in both hemispheres; however, there was a greater effect on the right amygdala. Regarding the left amygdala, there was an inverse association with the time of institutionalization (114). Institutionalization, even in the best conditions, is far from the context of typical care that has been established culturally for our species and seems to have consequences for emotional development impacting the macroscopic organization of our brain. In fact, an investigation carried out with institutionalized children showed the existence of differences in the volume of the amygdala, depending on whether the subjects were adopted early or late in life. Belatedly adopted children had significantly larger volumes of the amygdala than the volumes found in early adopted children and in those in the control group. There were no differences between those last two groups (115). From this perspective, it has been reported that mothers suffering from depression show a reduction in general sensitivity toward the infant, presenting a high rate of withdrawn behavior and precariousness in maternal care. It has been suggested that this behavior is analogous to the phenomenon of orphanhood, indirectly constituting a form of abuse due to negligence. When analyzing the structural changes in the brain of children who were taken care of by depressive mothers, specifically in the hippocampus and amygdala, no differences were found in relation to the hippocampus; however, there was a significant bilateral widening of the amygdala. This increase in the volume of the amygdala was directly correlated with the severity of the mother's depressive symptoms, as well as with elevated cortisol levels, compared with controls (116).

A reduction in the size of the left (7.9%) and of the right amygdala (7.5%) was found in women suffering BPD, who had had some type of childhood trauma due to abuse or neglect. This reduction, although important, was less than that described for the hippocampus, which was 16% (111). Moreover, another study showed a more significant decrease in the volume of the amygdala nucleus (21.9%) in women with BPD and a history of childhood abuse. However, this decrease was greater in the right amygdala (23%) than in the left (21%). The volumetric loss was greater in the amygdala than in the hippocampus (13.1%) (112). It was reported that the severity of symptoms was inversely associated with the bilateral size of the amygdala in subjects exposed to early stress and diagnosed with PTSD. When comparing the right and left amygdala volumes between subjects with PTSD diagnosis and undiagnosed patients who had a history of early stress, it was observed that the abused individuals without PTSD had a greater left amygdala volume (103).

In addition to the structural findings, investigations based on functional MRI have shown electrophysiological and hodological changes in the amygdala of subjects exposed to early stress. Such is the case of a study conducted in children who had a history of domestic abuse, which found increased cellular activity in the right amygdala after exposing subjects to face recognition paradigms with emotional value. Amygdala activation was inversely associated with the age of abuse onset. The authors suggested that the hyperactivity of the amygdala regarding the recognition of faces with emotional expressions, whether positive or negative, could be a neural prodrome for the later onset of a psychiatric disorder (117).

Regarding functional connectivity, it is known that in neurotypical development, the connections between the amygdala and the mPFC are immature in childhood and begin their refinement only in adolescence. When analyzing this connection in a group of previously institutionalized children who suffered maternal deprivation, a pattern of atypical connectivity was observed. This pattern was characterized by a level of maturation like that presented in adolescence. Additionally, it was observed that premature maturation of the circuit was directly associated with elevated cortisol levels, suggesting that the modifications in the connections could be mediated by the activity of the hypothalamic–pituitary–suprarenal axis. Similarly, the abused subjects had higher levels of anxiety, so it was hypothesized that prematurity in the maturation of the circuit may be a compensatory phenomenon in response to early adversity (118).

Some studies have suggested that the premature recruitment of the amygdala–mPFC network in childhood occurs only for stimuli with a negative value (119). The strength of the connections between the amygdala and the mPFC was evaluated in children aged between 6 and 10 years old with neurotypical development, in relation to the characteristics of parental care. The results of this work supported the idea that

the maturation of this network was related to the affective development of the individual. A significant effect was found with stronger connectivity in children with low scores in parental sensitivity. Gender analysis suggested a more marked effect on girls (120). From this same approach, functional changes in the amygdala–frontal connection were investigated during the processing of faces with negative emotional value, as well as their relationship with depressive signs in adolescents with a history of verbal abuse. The results showed that there was a positive correlation between the hyperactivation of the circuit established between the right amygdala and the anteroventral region of the cingulate gyrus in the abused patients. Similarly, aberrant activation of the right hemisphere was also associated with depressive symptoms. Together, these data were interpreted as latent mechanisms for the appearance of subsequent mood alterations (19).

Not all findings point in the same direction. In fact, some argue that the amygdala–mPFC functional connection is interrupted in infants suffering from early stress. When analyzing connectivity at rest in children aged 4–7 years old, a negative association was found between the number of adverse experiences and the activation of the amygdala–mPFC network. Besides, it was observed that this network hypoactivity was linked to higher levels of aggressive behaviors and attention failures (121). When examining the correlation between early stress and the genetic profiles of 10 polymorphisms of a nucleotide in genes associated with the HPA axis (CRHR1, NR3C2, NR3C1, and FKBP5) in school-age children (9–14 years old), investigators found that the greater the genetic risk, the greater the alterations to the functional connectivity between the amygdala and the caudate nucleus and the post-central gyrus. Exposure to early adverse and traumatic events was a predictor of a weak functional connection between the amygdala and the anterior cortex of the cingulate gyrus. Taken together, the sum of the genetic profile and the stress history predicted the attenuation of the network that links the amygdala and the inferior frontal gyrus, the middle frontal gyrus, the caudate nucleus, and the parahippocampal gyrus (140).

As in the case of the hippocampus (109, 134), studies suggest the existence of critical periods of vulnerability, and some authors propose a connoted impact in early childhood and prepuberty (109, 141).

Other Structures

In addition to the reported effects on brain structures such as the hippocampus, the amygdala, and the PFC, early exposure to stress also affects the organization, function, and connection of other brain structures. Some of the findings have been reported in relation to specific regions such as the insula, the cingulate gyrus, and the caudate nucleus, while other findings have been presented globally, referring, for example, to the volume of the intracranial parenchyma or the ventricular dimension. Such is the case of a study with children and adolescents diagnosed with PTSD and who had suffered early abuse, in which a significant reduction in the volume of intracranial gray matter was found. A ventricular widening was also reported, specifically of the frontal horn of the lateral ventricle (142). Another investigation whose objective was to inquire on the subsequent impact of early stress on brain volume in adolescents between 14 and 17 years old, found a decrease in gray matter volume, significantly related to the events that these subjects experienced before 5 years of age. This reduction was identified in subcortical structures such as the putamen, the caudate nucleus, and the thalamus, as well as in cortical regions such as the insula, the posterior region of the cingulate gyrus, the PFC, and the temporal lobe (143).

An analysis of the relationship between early stress, MDD, and structural changes in the brain was carried out with 3,036 participants. This study showed that increased exposure to childhood adversity was associated with a significant reduction in the volume of the caudate nuclei in women, independently of an MDD diagnosis. All types of adversity were negatively associated with the size of the caudate nucleus, predominantly emotional and physical abandonment (18). Findings like those of the previously mentioned study were also reported in another study; however, the impact on the caudate nucleus associated with early stress was observed in both men and women (144).

Another structure in which changes have been reported in relation to early stress is the cingulate gyrus, predominantly in the anterior region of this structure (ACC). Regarding the effect of different types of traumatic adverse events during childhood, one study found that those who had lost their father (or a first-degree blood relative), witnessed domestic violence, experienced sexual abuse, or had been harassed in childhood had a lower ACC volume (144). In another investigation with young adults who self-reported physical abuse in childhood, a volumetric reduction of 16.9% of the ACC was found. This decrease, like those of other structures assessed in that study, correlated significantly with the IQ measured on an intelligence scale (100). An investigation on adolescents with a diagnosis of PTSD secondary to childhood sexual abuse showed that they had significantly lower volumes in the dorsal portion of the ACC. The analysis of those findings allowed us to suggest a direct relationship between volumetric loss and child abuse, and not between the former and the pathophysiological mechanisms of PTSD (145).

Regarding the corpus callosum (CC), two studies with minors diagnosed with PTSD and a history of abuse showed a reduction in the median sagittal plane of sectors CC4, CC5, CC6, and CC7. This reduction extends from the middle portion of the body of the CC to the splenium. There was a tendency for the supragenual portion 2 to be smaller than the controls (142, 146). A study in children with a history of abuse and neglect compared the volume of their CC with that of children without such background and who may or may not had a psychiatric disorder. The total reduction in the CC volume in patients exposed to early stress reached 17% when compared with healthy controls, and 11% compared with those suffering from a psychiatric disorder. The type of traumatic event with the greatest impact associated with the reduction of the CC was negligence. This reduction was 15% in regions CC3, CC4, CC5, and 18% in CC7. Sexual abuse was the type of traumatic event with the greatest impact on girls

(147). In relation to the above, it has been reported that the effect on CC volume in women victims of recurrent sexual abuse during childhood could be greater when it is perpetrated when the child is between 9 and 10 years old (109). Other research carried out on young adults analyzed the impact of verbal abuse by peers during childhood on the CC, finding an increase in mean and radial diffusivity, and a decrease in fractional anisotropy and the radiated crown. In addition, there was a significant correlation between the degree of exposure to abuse and the average diffusivity of the splenium, as well as with the radial diffusivity of this same structure, that of the CC body, and that of the right posterior radiated crown. The findings did not show significant differences between sexes (148). It should be noted that not all studies on CC volumetry in individuals with a history of early stress have found changes in this structure (114).

Despite the tendency to describe changes in limbic structures, some studies have described variants in structures that do not seem to be directly related to the pathophysiological mechanisms associated with early stress. Such is the case of a study carried out on 23 non-medicated university women, who had a history of childhood sexual abuse. In this study, a reduction in the volume of cortical gray matter was found in areas BA17 and BA18, corresponding to the primary and associative visual areas, respectively. The reduction was 12.6% for the right hemisphere and 18.1% for the left one. In addition, a direct correlation was found between volumetric loss and chronicity of abuse before the age of 12 (149). Likewise, in subjects who witnessed domestic violence in childhood, a 6.1% reduction in the right lingual gyrus was found. There was also a reduction in the V2 area of both hemispheres and in the left occipital pole (150).

On the other hand, an investigation focused on the analysis of cortical areas involved in the processing of somatosensory information of the genital area, following the premise that women victims of child sexual abuse frequently report sexual dysfunction. This study, in addition to correlating the report of childhood adversity with an overall decrease in cortical gray matter, showed a significant effect on the left post-central gyrus (primary somatosensory area), specifically in the sectors where the clitoris is represented, and the area surrounding the genital and mouth regions (151). Another study of 24 children with a diagnosis of pediatric PTSD and a history of interpersonal trauma showed a significant reduction in the volume of the pons and of the posterior region of the cerebellar vermis (98).

In addition to volumetric changes in circumscribed regions of the gray cortical or subcortical matter, alterations in the anatomical connectivity between these structures has also been described in subjects exposed to early stress (152). In an analysis carried out with individuals who were exposed to verbal abuse in childhood, a significant loss of volume was found in the left arcuate fasciculus (AF) (at the level of the superior temporal gyrus), the cingulum bundle, and the body of the left fornix. Likewise, this analysis found that the decrease in the volume of AF region 1 was positively correlated with the level of maternal abuse, with IQ on verbal subscales, and with the verbal comprehension index. Meanwhile, volumetric changes in AF region 2 were inversely correlated with depression, dissociation, and emotional irritability. Finally, volumetric changes in AF region 3 were inversely correlated with somatization and anxiety scores (152). Another investigation that compared the density of the white matter of mentally healthy subjects with that of depressive subjects, with and without a history of abandonment in childhood, found that this density was significantly lower in the lower parietal lobe (bilateral) in subjects with a history of child neglect. In addition, there was an increase in the density of the extranuclear sublobar region (bilateral) and of the regions of the right midbrain, compared with those found in depressive subjects with no history of abuse. The findings for the inferior parietal lobule were negatively correlated with scores on the scales of childhood trauma, depression, and dysfunctional attitude used in the study (153). Significantly low values have been found for fractional anisotropy of the lateral region of the lower longitudinal fascicle in the left occipital lobe in subjects who witnessed domestic violence. The data of this investigation, particularly those of radial diffusivity, would suggest an alteration in the process of myelination of the occipitoparietal pathway, functionally considered as visuo-limbic and involved in emotional processing, learning, and visual memory, among others (154).

Regarding functional connectivity, one study compared patients diagnosed with MDD and reporting early trauma vs. patients diagnosed with MDD without early trauma vs. healthy subjects. That study found a generalized reduction in the activity of a circuit that linked the PFC, limbic structures, the thalamus, and the cerebellum in patients with a history of early trauma. Such weakening of the network was significantly correlated with high levels of childhood neglect (155). Another investigation that applied a paradigm of inhibitory control that activates a network underlying this function in subjects with a history of child abuse (both abuse and neglect) found changes in the functional connectivity of that functional network. This connectivity depended on the severity of exposure to child abuse (156). It has also been observed that exposure to visual stimuli with emotional value in adolescents with a history of early abuse generates hyperactivity in different nodes of the prominence network, having to do with the amygdala, the anterior region of the insula, and the putamen. This exacerbation of the response occurred specifically due to stimuli with negative emotional charge (157).

DISCUSSION

Over a 100 publications were reviewed for the present study; most corresponded to original research that evidenced the existence of a strong association between early life stress and changes in cognitive and behavioral activity, as well as with the presence of psychiatric disorders and alterations in the structure of the human brain.

Regarding functional alterations, reports assessing cognitive, neuropsychological, and behavioral activity, as well as associated psychiatric disorders were reviewed. Some of the studies collected showed a decrease in global intellectual functioning, affecting both the IQ and the level of intelligence. Other studies have shown specific changes in almost all neuropsychological

domains dependent on cortical activity, evidencing alterations in the higher mental functions required for language, visual-motor integration, instrumental and associative learning, various types of memory, visual and auditory attention, and executive functions. Alterations have also been found in several subdomains of mental processes, such as planning, problem-solving, attention, abstraction, reasoning, working memory, inhibitory control, decision making, and cognitive flexibility, all these in the field of executive functions. Although many studies have assessed cognitive and executive function alterations, very few studies have assessed the effect on school performance as well as on the level of general intelligence.

We have found sufficient and consistent evidence to suggest a cumulative effect of early life stress, which is directly associated with the severity of the alterations in the functions mentioned above, whose effects have been predominantly evaluated in adolescence and early adulthood, but that could extend beyond 60 years.

Some behavioral and psychopathological alterations also seem to have a strong relationship with childhood adversity. This is the case of the increased probability of use and/or abuse of psychoactive substances. Although we identified in the reviewed studies a strong association between negligence and emotional abuse with alcohol consumption, the truth is that all forms of early stress seem to increase the risk of consumption of alcohol and illicit drug use. Similarly, early stress is an effective predictor of psychiatric disorders, particularly depressive disorders and PTSD, as well as the development of ideation and suicidal acts. We also identified a tendency to strongly correlate suicidal ideation or suicide attempts with a history of sexual abuse; however, we lack a rigorous analysis to prove this claim since it was not one of the objectives of this review.

It is worth questioning the difficulty in discerning the effect that one type of stress may have on another, since it is common for those who suffer from early stress to be subjected to more than one type of stress. In this regard, most of the original studies collected in this review considered differential effects for each type of traumatic event in childhood.

In relation to the cerebral structural changes associated with early exposure to stress, we identified that the most studied human brain structures and for which there is a greater amount of data were the amygdala and the hippocampus, as can be seen by comparing **Tables 3**–**5**. Among the studies reviewed that reported changes in PFC and amygdala (see **Tables 3**, **5**), some were included whose main finding was an alteration in function or connectivity because these alterations were identified in specific brain structures. Despite the fact that these studies also presented associated behavioral or psychiatric alterations among the evaluated subjects, they were not included in the first section of the manuscript because the objective of these investigations was not to assess these alterations.

Regarding the hippocampus, there is a clear tendency to find a loss of its volume among those who suffered early stress; however, the data are not conclusive on whether this volumetric decrease affects it bilaterally or predominantly in the left hemisphere. It is also clear that further studies are needed to analyze specific differences for each subfield of the hippocampal formation, as well as changes in underlying molecular biomarkers using techniques such as SPECT. All studies that found a structural alteration of the hippocampus coincide in that the type of stress suffered corresponded to sexual and/or physical abuse; half of these were carried out only on women, and the most frequent behavioral or psychiatric disorders were PTSD, MDD, and BPD.

As for the amygdala, unlike the hippocampus, for which most of the publications collected indicated a decrease in volume, there was no consensus on a direction in its size change. There is more research evaluating the activity and functional connectivity of the amygdala than of the hippocampus or PFC (see **Tables 3**–**5**) and almost the same number of investigations evaluating structural changes. Among the studies that reported structural changes of the amygdala, there were no predominant types of associated behavioral or psychiatric disorders, and the most frequent factor was that these types of disorders are not reported or determined. The types of early stress experienced by subjects with structural changes in the amygdala are not the same types of stress associated with changes in the PFC or hippocampus (such as early institutional deprivation and family adversities).

There was also a good number of reports of structural changes in the PFC in subjects who suffered from early life stress; however, these were fewer than reports on the amygdala and hippocampus. Among these studies, there was no coincidence on a specific region of the PFC or on a direction (increase or decrease) in which the alteration occurred; however, in most studies, the structural change involved some sector of the medial surface of the PFC. There was no coincidence between these studies in the psychiatric, behavioral, or cognitive alterations associated with the structural changes, but there was some mood disorder in most of them. Structural changes to the PFC were associated with anxiety, social behavior, and attention disorders, which did not occur when the structural change occurred in the hippocampus or amygdala, such as phobias, the use of psychoactive substances, or ADHD. All the behavioral and psychiatric disorders associated with structural alterations to the PFC coincided in corresponding with the alteration to the functions attributed to the mPFC. The types of stress most frequently associated with structural changes in the PFC were, in descending order, emotional abuse, physical neglect, and emotional neglect.

In addition to the hippocampus, the amygdala, and the PFC (see **Figure 1**), we found changes in other regions of the cerebral gray and white substances. Such is the case of the insula, the anterior cingulate cortex, the occipital visual areas, the postcentral gyrus, the caudate nucleus, the putamen, the thalamus, the CC, the arcuate fascicle, the superior longitudinal fascicle, and the fornix. Despite the recognized involvement of the HPA in the process of stress consolidation, there is very little research that addresses this axis in humans, structurally or functionally.

In conclusion, there is strong evidence that the early stages of life constitute a critical period for the deleterious impact of adverse or traumatic situations. This evidence also suggests that adverse childhood experiences are associated not only with behavioral, cognitive, or neuropsychological changes but

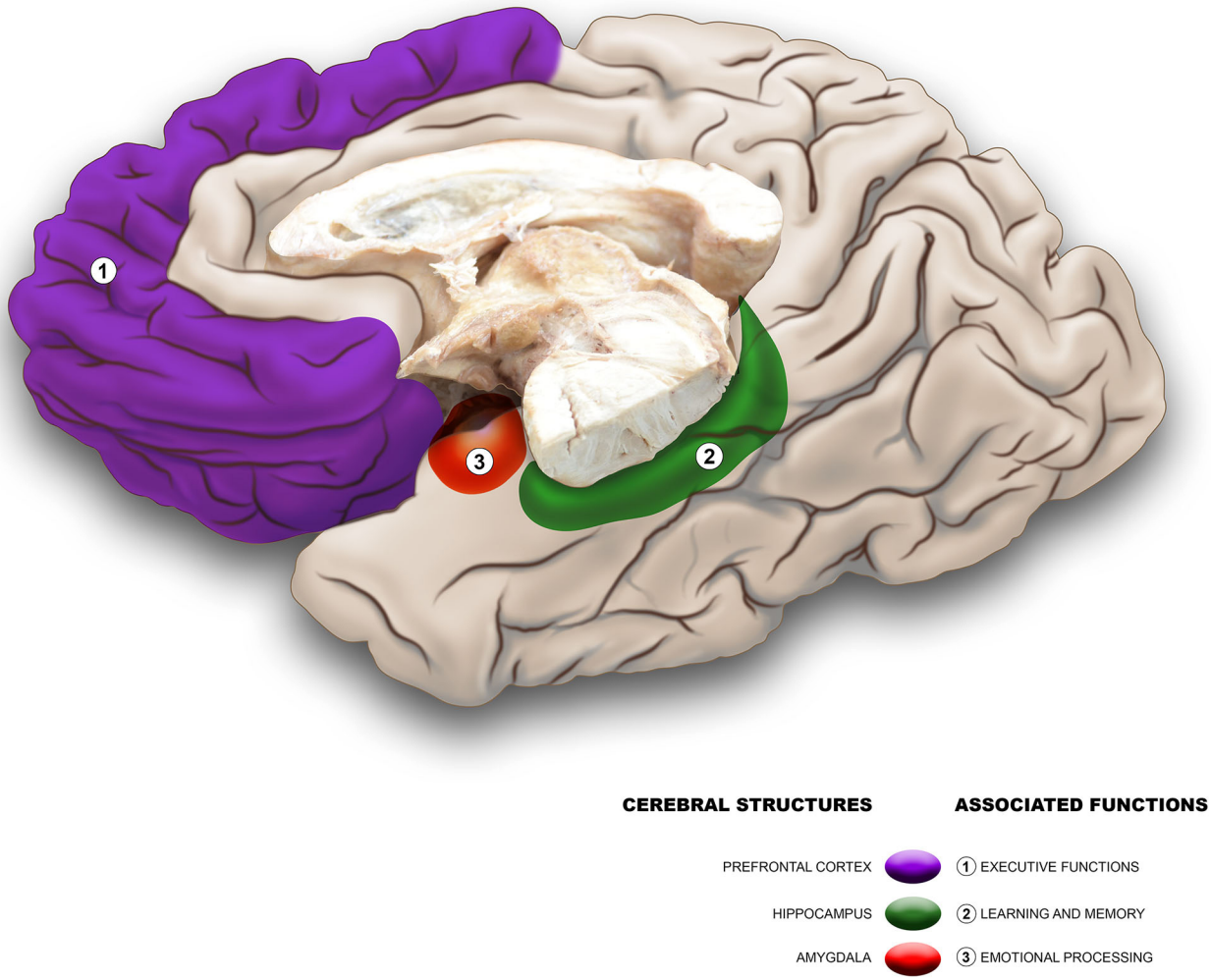

FIGURE 1 | Brain structures and mental functions with greatest number of alterations associated with early life stress.

also with psychiatric disorders and, even more so, that they could affect the anatomical and functional organization of brain structures such as the PFC, the hippocampus, and the amygdala. Similarly, studies in humans suggest that despite the deleterious effects of early stress, it is possible, at least in part, to lessen or reverse its consequences. Another very interesting aspect that must be taken into account when designing therapeutic strategies or public health policies refers to the fact that parental care seems to have a strong influence on the structural and functional organization of the human brain. Finally, from the studies that were included in this review, only a few were carried out in Latin American countries or other countries with limited economic resources. In the future, more research should point in this direction because there could be differential cerebral and behavioral effects of the types of stress experienced at early ages between different ethnic groups or between different socioeconomic conditions.

AUTHOR CONTRIBUTIONS

CG-A and EB contributed to conception and writing of the manuscript. CG-A carried out the review of the bibliographic bases. CG-A, CR-C, and EB reviewed the references collected and critically revised the manuscript. All authors approved the final manuscript.

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REFERENCES

1. Henríquez Santoya YI, Corredor Corvacho JÁ. Maltreatment and sexual abuse at home concerning schooling adolscents. *DUAZARY – Rev Fac Ciencias Salud.* (2011) 8:262–9. doi: 10.21676/2389783X.232
2. Santana-Tavira R, Sánchez-Ahedo R, Herrera-Basto E. El maltrato infantil: un problema mundial. *Salud Publica Mex.* (1998) 40:58–65. doi: 10.1590/S0036-36341998000100009
3. Herrera-Basto E. Indicadores para la deteccion de maltrato en ninos. *Salud Publica Mex.* (1999) 41:420–5. doi: 10.1590/S0036-36341999000500011
4. Claussen AH, Crittenden PM. Physical and psychological maltreatment: relations among types of maltreatment. *Child Abus Negl.* (1991) 15:5–18. doi: 10.1016/0145-2134(91)90085-R
5. Organization WH. *Child Maltreatment and Alcohol Fact Sheet*. Geneve-Suisse: World Health Organization (2005).
6. Martínez GS. El maltrato infantil: mecanismos subyacentes. *Av Psicol Latinoam*. (2008) 26:171–9.
7. García Ruiz MM. Violencia intrafamiliar, Colombia 2001. *Forensis Datos Para Vida*. (2001) 1:88–127.
8. Sierra Fajardo RA, Macana Tuta NL, Cortés Callejas C. Impacto social de la violencia intrafamiliar. *Forensis Datos Para Vida*. (2006) 1:81–90.
9. Felitti VJ. The relation between adverse childhood experiences and adult health: turning gold into lead. *Perm J.* (2002) 6:44–7.
10. Arnsten AFT. Stress signalling pathways that impair prefrontal cortex structure and function. *Nat Rev Neurosci.* (2009) 10:410–22. doi: 10.1038/nrn2648
11. Dube SR, Felitti VJ, Dong M, Chapman DP, Giles WH, Anda RF. Childhood abuse, neglect, and household dysfunction and the risk of illicit drug use: the adverse childhood experiences study. *Pediatrics.* (2003) 111:564–72. doi: 10.1542/peds.111.3.564
12. Hillis SD, Anda RF, Felitti VJ, Nordenberg D, Marchbanks PA. Adverse childhood experiences and sexually transmitted diseases in men and women: a retrospective study. *Pediatrics.* (2000) 106:1–6. doi: 10.1542/peds.106.1.e11
13. Dietz PM, Spitz AM, Anda RF, Williamson DF, McMahon PM, Santelli JS, et al. Unintended pregnancy among adult women exposed to abuse or household dysfunction during their childhood. *JAMA.* (1999) 282:1359–64. doi: 10.1001/jama.282.14.1359
14. Nanni V, Uher R, Danese A. Childhood maltreatment predicts unfavorable course of illness and treatment outcome in depression: a meta-analysis. *Am J Psychiatry.* (2012) 169:141–51. doi: 10.1176/appi.ajp.2011.11020335
15. Beers SR, Bellis MDD. Neuropsychological function in children with maltreatment-related posttraumatic stress disorder. *Am J Psychiatry.* (2002) 159:483–6. doi: 10.1176/appi.ajp.159.3.483
16. Lupien SJ, McEwen BS, Gunnar MR, Heim C. Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nat Rev Neurosci.* (2009) 10:434–45. doi: 10.1038/nrn2639
17. Mezzacappa E, Kindlon D, Earls F. Child abuse and performance task assessments of executive functions in boys. *J Child Psychol Psychiatry Allied Discip.* (2001) 42:1041–8. doi: 10.1111/1469-7610.00803
18. Frodl T, Janowitz D, Schmaal L, Tozzi L, Dobrowolny H, Stein DJ, et al. Childhood adversity impacts on brain subcortical structures relevant to depression. *J Psychiatr Res.* (2017) 86:58–65. doi: 10.1016/j.jpsychires.2016.11.010
19. Lee SW, Yoo JH, Kim KW, Lee JS, Kim D, Park H, et al. Aberrant function of frontoamygdala circuits in adolescents with previous verbal abuse experiences. *Neuropsychologia.* (2015) 79:76–85. doi: 10.1016/j.neuropsychologia.2015.10.029
20. Teicher MH, Andersen SL, Polcari A, Anderson CM, Navalta CP, Kim DM. The neurobiological consequences of early stress and childhood maltreatment. *Neurosci Biobehav Rev.* (2003) 27:33–44. doi: 10.1016/S0149-7634(03)00007-1
21. Hanson JL, Chung MK, Avants BB, Shirtcliff EA, Gee JC, Davidson RJ, et al. Early stress is associated with alterations in the orbitofrontal cortex: a tensor-based morphometry investigation of brain structure and behavioral risk. *J Neurosci.* (2010) 30:7466–72. doi: 10.1523/JNEUROSCI.0859-10.2010
22. Davidson RJ, McEwen BS. Social influences on neuroplasticity: stress and interventions to promote well-being. *Nat Neurosci.* (2012) 15:689–95. doi: 10.1038/nn.3093
23. Mercy JA, Saul J. Creating a healthier future through early interventions for children. *J Am Med Assoc.* (2009) 301:2262–4. doi: 10.1001/jama.2009.803
24. Berkowitz SJ, Stover CS, Marans SR. The child and family traumatic stress intervention: secondary prevention for youth at risk of developing PTSD. *J Child Psychol Psychiatry Allied Discip.* (2011) 52:676–85. doi: 10.1111/j.1469-7610.2010.02321.x
25. Walker SP, Wachs TD, Grantham-McGregor S, Black MM, Nelson CA, Huffman SL, et al. Inequality in early childhood: risk and protective factors for early child development. *Lancet.* (2011) 378:1325–38. doi: 10.1016/S0140-6736(11)60555-2
26. Cohen JA, Mannarino AP, Iyengar S. Community treatment of posttraumatic stress disorder for children exposed to intimate partner violence: a randomized controlled trial. *Arch Pediatr Adolesc Med.* (2011) 165:16–21. doi: 10.1001/archpediatrics.2010.247
27. Fisher PA, Gunnar MR, Dozier M, Bruce J, Pears KC. Effects of therapeutic interventions for foster children on behavioral problems, caregiver attachment, and stress regulatory neural systems. *Ann N Y Acad Sci.* (2006) 1094:215–25. doi: 10.1196/annals.1376.023
28. McEwen BS. Physiology and neurobiology of stress and adaptation: central role of the brain. *Physiol Rev.* (2007) 87:873–904. doi: 10.1152/physrev.00041.2006
29. Sandi C, Haller J. Stress and the social brain: behavioural effects and neurobiological mechanisms. *Nat Rev Neurosci.* (2015) 16:290–304. doi: 10.1038/nrn3918
30. Ulrich-Lai YM, Herman JP. Neural regulation of endocrine and autonomic stress responses. *Nat Rev Neurosci.* (2009) 10:397–409. doi: 10.1038/nrn2647
31. van Bodegom M, Homberg JR, Henckens MJAG. Modulation of the hypothalamic-pituitary-adrenal axis by early life stress exposure. *Front Cell Neurosci.* (2017) 11:87. doi: 10.3389/fncel.2017.00087
32. Steckler T, Holsboer F. Corticotropin-releasing hormone receptor subtypes and emotion. *Biol Psychiatry.* (1999) 46:1480–508. doi: 10.1016/S0006-3223(99)00170-5
33. Arzt E, Holsboer F. CRF signaling: molecular specificity for drug targeting in the CNS. *Trends Pharmacol Sci.* (2006) 27:531–8. doi: 10.1016/j.tips.2006.08.007
34. Starkman MN, Gebarski SS, Berent S, Schteingart DE. Hippocampal formation volume, memory dysfunction, and cortisol levels in patients with Cushing's syndrome. *Biol Psychiatry.* (1992) 32:756–65. doi: 10.1016/0006-3223(92)90079-F
35. Lupien SJ, de Leon M, de Santi S, Convit A, Tarshish C, Nair NPV, et al. Cortisol levels during human aging predict hippocampal atrophy and memory deficits. *Nat Neurosci.* (1998) 1:69–73. doi: 10.1038/271
36. McEwen BS. Stress and hippocampal plasticity. *Annu Rev Neurosci.* (1999) 22:105–22. doi: 10.1146/annurev.neuro.22.1.105
37. Magariños AM, García Verdugo JM, McEewen BS. Chronic stress alters synaptic terminal structure in hippocampus. *Proc Natl Acad Sci USA.* (1997) 94:14002–8. doi: 10.1073/pnas.94.25.14002
38. Rowe E, Eckenrode J. The timing of academic difficulties among maltreated and nonmaltreated children. *Child Abus Negl.* (1999) 23:813–32. doi: 10.1016/S0145-2134(99)00044-7
39. Bremner JD, Randall P, Scott TM, Capelli S, Delaney R, McCarthy G, et al. Deficits in short-term memory in adult survivors of childhood abuse. *Psychiatry Res.* (1995) 59:97–107. doi: 10.1016/0165-1781(95)02800-5
40. Stein MB, Hanna C, Vaerum V, Koverola C. Memory functioning in adult women traumatized by childhood sexual abuse. *J Trauma Stress.* (1999) 12:527–34. doi: 10.1023/A:1024775222098
41. Saigh PA, Yasik AE, Oberfield RA, Halamandaris PV, Bremner JD. The intellectual performance of traumatized children and adolescents with or without posttraumatic stress disorder. *J Abnorm Psychol.* (2006) 115:332–40. doi: 10.1037/0021-843X.115.2.332
42. Huth-Bocks AC, Levendosky AA, Semel MA. The direct and indirect effects of domestic violence on young children's intellectual functioning. *J Fam Violence.* (2001) 16:269–90. doi: 10.1023/A:1011138332712
43. Saltzman KM, Weems CF, Carrion VG. IQ and posttraumatic stress symptoms in children exposed to interpersonal violence. *Child Psychiatry Hum Dev.* (2006) 36:261–72. doi: 10.1007/s10578-005-0002-5

44. Nadeau ME, Nolin P. Attentional and executive functions in neglected children. *J Child Adolesc Trauma.* (2013) 6:1–10. doi: 10.1080/19361521.2013.733794
45. De Bellis MD, Hooper SR, Spratt EG, Woolley DP. Neuropsychological findings in childhood neglect and their relationships to pediatric PTSD. *J Int Neuropsychol Soc.* (2009) 15:868–78. doi: 10.1017/S1355617709990464
46. De Bellis MD, Woolley DP, Hooper SR. Neuropsychological findings in pediatric maltreatment: relationship of PTSD, dissociative symptoms, and abuse/neglect indices to neurocognitive outcomes. *Child Maltreat.* (2013) 18:171–83. doi: 10.1177/1077559513497420
47. Hostinar CE, Stellern SA, Schaefer C, Carlson SM, Gunnar MR. Associations between early life adversity and executive function in children adopted internationally from orphanages. *Proc Natl Acad Sci USA.* (2012) 109(Suppl. 2):17208–12. doi: 10.1073/pnas.1121246109
48. Navalta CP, Polcari A, Webster DM, Boghossian A, Teicher MH. Effects of childhood sexual abuse on neuropsychological and cognitive function in college women. *J Neuropsychiatry Clin Neurosci.* (2006) 18:45–53. doi: 10.1176/jnp.18.1.45
49. Cicchetti D, Rogosch FA, Howe ML, Toth SL. The effects of maltreatment and neuroendocrine regulation on memory performance. *Child Dev.* (2010) 81:1504–19. doi: 10.1111/j.1467-8624.2010.01488.x
50. Samuelson KW, Krueger CE, Burnett C, Wilson CK. Neuropsychological functioning in children with posttraumatic stress disorder. *Child Neuropsychol.* (2010) 16:119–33. doi: 10.1080/09297040903190782
51. Bremner JD, Vermetten E, Afzal N, Vythilingam M. Deficits in verbal declarative memory function in women with childhood sexual abuse-related posttraumatic stress disorder. *J Nerv Ment Dis.* (2004) 192:643–9. doi: 10.1097/01.nmd.0000142027.52893.c8
52. Jouriles EN, Brown AS, McDonald R, Rosenfield D, Leahy MM, Silver C. Intimate partner violence and preschoolers' explicit memory functioning. *J Fam Psychol.* (2008) 22:420–8. doi: 10.1037/0893-3200.22.3.420
53. Hanson JL, van den Bos W, Roeber BJ, Rudolph KD, Davidson RJ, Pollack SD. Early adversity and learning: implications for typical and atypical behavioral development. *J Child Psychol Psychiatry Allied Discip.* (2017) 58:770–8. doi: 10.1111/jcpp.12694
54. Harms MB, Shannon Bowen KE, Hanson JL, Pollak SD. Instrumental learning and cognitive flexibility processes are impaired in children exposed to early life stress. *Dev Sci.* (2018) 21:e12596. doi: 10.1111/desc.12596
55. Nolin P, Ethier L. Using neuropsychological profiles to classify neglected children with or without physical abuse. *Child Abus Negl.* (2007) 31:631–43. doi: 10.1016/j.chiabu.2006.12.009
56. Bücker J, Kapczinski F, Post R, Ceresér KM, Szobot C, Yatham LN, et al. Cognitive impairment in school-aged children with early trauma. *Compr Psychiatry.* (2012) 53:758–64. doi: 10.1016/j.comppsych.2011.12.006
57. Perna RB, Kiefner M. Long-term cognitive sequelae: abused children without PTSD. *Appl Neuropsychol Child.* (2013) 2:1–5. doi: 10.1080/09084282.2011.595460
58. Barrera M, Calderón L, Bell V. The cognitive impact of sexual abuse and PTSD in children: a neuropsychological study. *J Child Sex Abus.* (2013) 22:625–38. doi: 10.1080/10538712.2013.811141
59. Hanson JL, Adluru N, Chung MK, Alexander AL, Davidson RJ, Pollak SD. Early neglect is associated with alterations in white matter integrity and cognitive functioning. *Child Dev.* (2013) 84:1566–78. doi: 10.1111/cdev.12069
60. Roos LE, Giuliano RJ, Beauchamp KG, Berkman ET, Knight EL, Fisher PA. Acute stress impairs children's sustained attention with increased vulnerability for children of mothers reporting higher parenting stress. *Dev Psychobiol.* (2019) 62:532–43. doi: 10.1002/dev.21915
61. DePrince AP, Weinzierl KM, Combs MD. Executive function performance and trauma exposure in a community sample of children. *Child Abus Negl.* (2009) 33:353–61. doi: 10.1016/j.chiabu.2008.08.002
62. Lamm C, Troller-Renfree SV, Zeanah CH, Nelson CA, Fox NA. Impact of early institutionalization on attention mechanisms underlying the inhibition of a planned action. *Neuropsychologia.* (2018) 117:339–46. doi: 10.1016/j.neuropsychologia.2018.06.008
63. Weller JA, Fisher PA. Decision-making deficits among maltreated children. *Child Maltreat.* (2013) 18:184–94. doi: 10.1177/1077559512467846
64. Hanson JL, Chung MK, Avants BB, Rudolph KD, Shirtcliff EA, Gee JC, et al. Structural variations in prefrontal cortex mediate the relationship between early childhood stress and spatial working memory. *J Neurosci.* (2012) 32:7917–25. doi: 10.1523/JNEUROSCI.0307-12.2012
65. Jacobs JR, Bovasso GB. Early and chronic stress and their relation to breast cancer. *Psychol Med.* (2000) 30:669–78. doi: 10.1017/S0033291799002020
66. Anda RF, Felitti VJ, Bremner JD, Walker JD, Whitfield C, Perry BD, et al. The enduring effects of abuse and related adverse experiences in childhood: a convergence of evidence from neurobiology and epidemiology. *Eur Arch Psychiatry ans Clin Neurosci.* (2006) 256:174–86. doi: 10.1007/s00406-005-0624-4
67. Hostinar CE, Nusslock R, Miller GE. Future directions in the study of early-life stress and physical and emotional health: implications of the neuroimmune network hypothesis. *J Clin Child Adolesc Psychol.* (2018) 47:142–56. doi: 10.1080/15374416.2016.1266647
68. Taylor SE. Mechanisms linking early life stress to adult health outcomes. *Proc Natl Acad Sci USA.* (2010) 107:8507–12. doi: 10.1073/pnas.1003890107
69. Ferraro KF, Schafer MH, Wilkinson LR. Childhood disadvantage and health problems in middle and later life: early imprints on physical health? *Am Sociol Rev.* (2016) 81:107–33. doi: 10.1177/0003122415619617
70. Felitti VJ, Anda RF, Nordenberg D, Williamson DF, Spitz AM, Edwards V, et al. Relationship of childhood abuse and household dysfunction to many of the leading causes of death in adults: the adverse childhood experiences (ACE) study. *Am J Prev Med.* (1998) 14:245–58. doi: 10.1016/S0749-3797(98)00017-8
71. Ouellet-Morin I, Odgers CL, Danese A, Bowes L, Shakoor S, Papadopoulos AS, et al. Blunted cortisol responses to stress signal social and behavioral problems among maltreated/bullied 12-year-old children. *Biol Psychiatry.* (2011) 70:1–18. doi: 10.1016/j.biopsych.2011.06.017
72. Hanson JL, Nacewicz BM, Sutterer MJ, Cayo AA, Schaefer SM, Rudolph KD, et al. Behavioral problems after early life stress: contributions of the hippocampus and amygdala. *Biol Psychiatry.* (2015) 77:314–23. doi: 10.1016/j.biopsych.2014.04.020
73. Deykin EY, Alpert JJ, Mcnamarra JJ. A pilot study of the effect of exposure to child abuse or neglect on adolescent suicidal behavior. *Am J Psychiatry.* (1985) 142:1299–303. doi: 10.1176/ajp.142.11.1299
74. Medrano MA, Zule WA, Hatch J, Desmond DP. Prevalence of childhood trauma in a community sample of substance-abusing women. *Am J Drug Alcohol Abuse.* (1999) 25:449–62. doi: 10.1081/ADA-100101872
75. Khoury L, Tang YL, Bradley B, Cubells JF, Ressler KJ. Substance use, childhood traumatic experience, and posttraumatic stress disorder in an urban civilian population. *Depress Anxiety.* (2010) 27:1077–86. doi: 10.1002/da.20751
76. Huang S, Trapido E, Fleming L, Arheart K, Crandall L, French M, et al. The long-term effects of childhood maltreatment experiences on subsequent illicit drug use and drug-related problems in young adulthood. *Addict Behav.* (2011) 36:95–102. doi: 10.1016/j.addbeh.2010.09.001
77. Widom CS, Marmorstein NR, White HR. Childhood victimization and illicit drug use in middle adulthood. *Psychol Addict Behav.* (2006) 20:394–403. doi: 10.1037/0893-164X.20.4.394
78. Schwandt ML, Heilig M, Hommer DW, George DT, Ramchandani VA. Childhood trauma exposure and alcohol dependence severity in adulthood: mediation by emotional abuse severity and neuroticism. *Alcohol Clin Exp Res.* (2013) 37:984–92. doi: 10.1111/acer.12053
79. Lotzin A, Haupt L, von Schönfels J, Wingenfeld K, Schäfer I. Profiles of childhood trauma in patients with alcohol dependence and their associations with addiction-related problems. *Alcohol Clin Exp Res.* (2016) 40:543–52. doi: 10.1111/acer.12990
80. Dube SR, Anda RF, Felitti VJ, Edwards VJ, Croft JB. Adverse childhood experiences and personal alcohol abuse as an adult. *Addict Behav.* (2002) 27:713–25. doi: 10.1016/S0306-4603(01)00204-0
81. Hyman SM, Garcia M, Sinha R. Gender specific associations between types of childhood maltreatment and the onset, escalation and severity of substance use in cocaine dependent adults. *Am J Drug Alcohol Abuse.* (2006) 32:655–64. doi: 10.1080/10623320600919193
82. Evren C, Evren B, Dalbudak E, Ozcelik B, Oncu F. Childhood abuse and neglect as a risk factor for alexithymia in adult male

substance dependent inpatients. *J Psychoactive Drugs.* (2009) 41:85–92. doi: 10.1080/02791072.2009.10400677
83. Frodl T, Reinhold E, Koutsouleris N, Reiser M, Meisenzahl EM. Interaction of childhood stress with hippocampus and prefrontal cortex volume reduction in major depression. *J Psychiatr Res.* (2010) 44:799–807. doi: 10.1016/j.jpsychires.2010.01.006
84. Seok JH, Lee KU, Kim W, Lee SH, Kang EH, Ham BJ, et al. Impact of early-life stress and resilience on patients with major depressive disorder. *Yonsei Med J.* (2012) 53:1093–8. doi: 10.3349/ymj.2012.53.6.1093
85. Chapman DP, Whitfield CL, Felitti VJ, Dube SR, Edwards VJ, Anda RF. Adverse childhood experiences and the risk of depressive disorders in adulthood. *J Affect Disord.* (2004) 82:217–25. doi: 10.1016/j.jad.2003.12.013
86. Shapero BG, Black SK, Liu RT, Klugman J, Bender RE, Abramson LY, et al. Stressful life events and depression symptoms: the effect of childhood emotional abuse on stress reactivity. *J Clin Psychol.* (2014) 70:209–23. doi: 10.1002/jclp.22011
87. Martins-Monteverde CMS, Baes CVW, Reisdorfer E, Padovan T, Tofoli SMDC, Juruena MF. Relationship between depression and subtypes of early life stress in adult psychiatric patients. *Front Psychiatry.* (2019) 10:19. doi: 10.3389/fpsyt.2019.00019
88. Bahk YC, Jang SK, Choi KH, Lee SH. The relationship between childhood trauma and suicidal ideation: role of maltreatment and potential mediators. *Psychiatry Investig.* (2017) 14:37–43. doi: 10.4306/pi.2017.14.1.37
89. Plunkett A, O'Toole B, Swanston H, Oates RK, Shrimpton S, Parkinson P. Suicide risk following child sexual abuse. *Ambul Pediatr.* (2001) 1:262–6. doi: 10.1367/1539-4409 (2001) 001<0262:SRFCSA>2.0.CO;2
90. Spokas M, Wenzel A, Stirman SW, Brown GK, Beck AT. Suicide risk factors and mediators between childhood sexual abuse and suicide ideation among male and female suicide attempters. *J Trauma Stress.* (2009) 22:467–70. doi: 10.1002/jts.20438
91. Brezo J, Paris J, Vitaro F, Hébert M, Tremblay RE, Turecki G. Predicting suicide attempts in young adults with histories of childhood abuse. *Br J Psychiatry.* (2008) 193:134–9. doi: 10.1192/bjp.bp.107.037994
92. Rabinovitch SM, Kerr DCR, Leve LD, Chamberlain P. Suicidal behavior outcomes of childhood sexual abuse: longitudinal study of adjudicated girls. *Suicide Life Threat Behav.* (2015) 45:431–47. doi: 10.1111/sltb.12141
93. Dube SR, Anda RF, Felitti VJ, Chapman DP, Williamson DF, Giles WH. Childhood abuse, household dysfunction, and the risk of attempted suicide throughout the life span: findings from the adverse childhood experiences study. *JAMA.* (2001) 286:3089–96. doi: 10.1001/jama.286.24.3089
94. Ystgaard M, Hestetun I, Loeb M, Mehlum L. Is there a specific relationship between childhood sexual and physical abuse and repeated suicidal behavior? *Child Abus Negl.* (2004) 28:863–75. doi: 10.1016/j.chiabu.2004.01.009
95. Porto Barbosa L, Quevedo L, Del Grande da Silva G, Jansen K, Tavares Pinheiro R, Branco J, et al. Childhood trauma and suicide risk in a sample of young individuals aged 14-35 years in southern Brazil. *Child Abus Negl.* (2014) 38:1191–6. doi: 10.1016/j.chiabu.2014.02.008
96. Behr Gomes Jardim G, Novelo M, Spanemberg L, von Gunten A, Engroff P, Lopes Nogueira E, et al. Influence of childhood abuse and neglect subtypes on late-life suicide risk beyond depression. *Child Abus Negl.* (2018) 80:249–56. doi: 10.1016/j.chiabu.2018.03.029
97. Richert KA, Carrion VG, Karchemskiy A, Reiss AL. Regional differences of the prefrontal cortex in pediatric PTSD: an MRI study. *Depress Anxiety.* (2006) 23:17–25. doi: 10.1002/da.20131
98. Carrion VG, Weems CF, Watson C, Eliez S, Menon V, Reiss AL. Converging evidence for abnormalities of the prefrontal cortex and evaluation of midsagittal structures in pediatric posttraumatic stress disorder: an MRI study. *Psychiatry Res Neuroimaging.* (2009) 172:226–34. doi: 10.1016/j.pscychresns.2008.07.008
99. Van Harmelen A, Van Tol M, Van Der Wee NJA, Veltman DJ, Aleman A, Spinhoven P, et al. Reduced medial prefrontal cortex volume in adults peporting childhood emotional maltreatment. *Biol Psychiatry.* (2010) 68:832–8. doi: 10.1016/j.biopsych.2010.06.011
100. Tomoda A, Suzuki H, Rabi K, Sheu Y, Polcari A, Teicher MH. Reduced prefrontal cortical gray matter volume in young adults exposed to harsh corporal punishment. *Neuroimage.* (2009) 47:T66–71. doi: 10.1016/j.neuroimage.2009.03.005
101. Van Harmelen A, Tol V, Dalgleish T, Van Der Wee NJA, Veltman DJ, Spinhoven P, et al. Hypoactive medial prefrontal cortex functioning in adults reporting childhood emotional maltreatment. *Scan.* (2014) 9:2026–33. doi: 10.1093/scan/nsu008
102. Van Harmelen A, Hauber K, Moor BG, Spinhoven P, Boon AE, Crone EA, et al. Childhood emotional maltreatment severity is associated with dorsal medial prefrontal cortex responsivity to social exclusion in young adults. *PLoS ONE.* (2014) 9:e85107. doi: 10.1371/journal.pone.0085107
103. Morey RA, Haswell CC, Hooper SR, De Bellis MD. Amygdala, hippocampus, and ventral medial prefrontal cortex volumes differ in maltreated youth with and without chronic posttraumatic stress disorder. *Neuropsychopharmacology.* (2016) 41:791–801. doi: 10.1038/npp.2015.205
104. Carrion VG, Weems CF, Reiss AL. Stress predicts brain changes in children: a pilot longitudinal study on youth stress, posttraumatic stress disorder, and the hippocampus. *Pediatrics.* (2007) 119:509–16. doi: 10.1542/peds.2006-2028
105. Vythilingam M, Heim C, Newport J, Miller AH, Anderson E, Bronen R, et al. Childhood trauma associated with smaller hippocampal volume in women with major depression. *Am J Psychiatry.* (2002) 159:2072–80. doi: 10.1176/appi.ajp.159.12.2072
106. Teicher MH, Anderson CM, Polcari A. Childhood maltreatment is associated with reduced volume in the hippocampal subfields CA3, dentate gyrus, and subiculum. *Proc Natl Acad Sci USA.* (2012) 109:E563–72. doi: 10.1073/pnas.1115396109
107. Rao U, Chen LA, Bidesi AS, Shad MU, Thomas MA, Hammen CL. Hippocampal changes associated with early-life adversity and vulnerability to depression. *Biol Psychiatry.* (2010) 67:357–64. doi: 10.1016/j.biopsych.2009.10.017
108. Bremner JD, Vythilingam M, Vermetten E, Southwick SM, McGlashan T, Nazeer A, et al. MRI and PET study of deficits in hippocampal structure and function in women with childhood sexual abuse and posttraumatic stress disorder. *Am J Psychiatry.* (2003) 160:924–32. doi: 10.1176/appi.ajp.160.5.924
109. Andersen SL, Tomoda A, Vincow ES, Valente E, Polcari A, Teicher MH. Preliminary evidence for sensitive periods in the effect of childhood sexual abuse on regional brain development. *J Neuropsychiatry Clin Neurosci.* (2008) 20:292–301. doi: 10.1176/jnp.2008.20.3.292
110. Bremner JD, Randall P, Vermetten E, Staib L, Bronen RA, Mazure C, et al. Magnetic resonance imaging-based measurement of hippocampal volume in posttraumatic stress disorder related to childhood physical and sexual abuse - A preliminary report. *Biol Psychiatry.* (1997) 41:23–32. doi: 10.1016/S0006-3223(96)00162-X
111. Driessen M, Herrmann J, Stahl K, Zwaan M, Meier S, Hill A, et al. Magnetic resonance imaging volumes of the hippocampus and the amygdala in women with borderline personality disorder and early traumatization. *Arch Gen Psychiatry.* (2000) 57:1115–22. doi: 10.1001/archpsyc.57.12.1115
112. Schmahl CG, Vermetten E, Elzinga BM, Bremner JD. Magnetic resonance imaging of hippocampal and amygdala volume in women with childhood abuse and borderline personality disorder. *Psychiatry Res Neuroimaging.* (2003) 122:193–8. doi: 10.1016/S0925-4927(03)00023-4
113. McLaughlin KA, Sheridan MA, Gold AL, Duys A, Lambert HK, Peverill M, et al. Maltreatment exposure, brain structure, and fear conditioning in children and adolescents. *Neuropsychopharmacology.* (2016) 41:1956–64. doi: 10.1038/npp.2015.365
114. Mehta MA, Golembo NI, Nosarti C, Colvert E, Mota A, Williams SCR, et al. Amygdala, hippocampal and corpus callosum size following severe early institutional deprivation: the english and romanian adoptees study pilot. *J Child Psychol Psychiatry.* (2009) 50:943–51. doi: 10.1111/j.1469-7610.2009.02084.x
115. Tottenham N, Hare TA, Quinn BT, McCarry TW, Nurse M, Gilhooly T, et al. Prolonged institutional rearing is associated with atypically large amygdala volume and difficulties in emotion regulation. *Dev Sci.* (2010) 13:46–61. doi: 10.1111/j.1467-7687.2009.00852.x
116. Lupien SJ, Parent S, Evans AC, Tremblay RE, Zelazo PD, Corbo V, et al. Larger amygdala but no change in hippocampal volume in 10-year-old children exposed to maternal depressive symptomatology since birth. *Proc Natl Acad Sci USA.* (2011) 108:14324–9. doi: 10.1073/pnas.1105371108
117. McCrory EJ, De Brito SA, Kelly PA, Bird G, Sebastian CL, Mechelli A, et al. Amygdala activation in maltreated children

during pre-attentive emotional processing. *Br J Psychiatry.* (2013) 202:269–76. doi: 10.1192/bjp.bp.112.116624
118. Gee DG, Gabard-Durnam LJ, Flannery J, Goff B, Humphreys KL, Telzer EH, et al. Early developmental emergence of human amygdala-prefrontal connectivity after maternal deprivation. *Proc Natl Acad Sci USA.* (2013) 110:15638–43. doi: 10.1073/pnas.1307893110
119. Herringa RJ, Burghy CA, Stodola DE, Fox ME, Davidson RJ, Essex MJ. Enhanced prefrontal-amygdala connectivity following childhood adversity as a protective mechanism against internalizing in adolescence. *Biol Psychiatry Cogn Neurosci Neuroimaging.* (2016) 1:326–34. doi: 10.1016/j.bpsc.2016.03.003
120. Thijssen S, Muetzel RL, Bakermans-Kranenburg MJ, Jaddoe VWV, Tiemeier H, Verhulst FC, et al. Insensitive parenting may accelerate the development of the amygdala-medial prefrontal cortex circuit. *Dev Psychopathol.* (2017) 29:505–18. doi: 10.1017/S0954579417000141
121. Park AT, Leonard JA, Saxler PK, Cyr AB, Gabrieli JDE, Mackey AP. Amygdala-medial prefrontal cortex connectivity relates to stress and mental health in early childhood. *Soc Cogn Affect Neurosci.* (2018) 13:430–9. doi: 10.1093/scan/nsy017
122. Goldman-Rakic PS. The prefrontal landscape: implications of functional architecture for understanding human mentation and the central executive. *Philos Trans R Soc Lond B Biol Sci.* (1996) 351:1445–53. doi: 10.1098/rstb.1996.0129
123. Thompson-Schill SL, Jonides J, Marshuetz C, Smith EE, D'Esposito M, Kan IP, et al. Effects of frontal lobe damage on interference effects in working memory. *Cogn Affect Behav Neurosci.* (2002) 2:109–20. doi: 10.3758/CABN.2.2.109
124. Aron AR, Robbins TW, Poldrack RA. Inhibition and the right inferior frontal cortex. *Trends Cogn Sci.* (2004) 8:170–7. doi: 10.1016/j.tics.2004.02.010
125. Buschman TJ, Miller EK. Top-down versus bottom-up control of attention in the preforntal and posterior parietal cortices. *Science.* (2007) 315:1860–2. doi: 10.1126/science.1138071
126. Lee D, Seo H. Mechanisms of reinforcement learning. *Ann New York Acad Sci.* (2007) 122:108–22. doi: 10.1196/annals.1390.007
127. Schweizer S, Walsh ND, Stretton J, Dunn VJ, Goodyer IM, Dalgleish T. Enhanced emotion regulation capacity and its neural substrates in those exposed to moderate childhood adversity. *Soc Cogn Affect Neurosci.* (2016) 11:272–81. doi: 10.1093/scan/nsv109
128. Cisler JM. Childhood trauma and functional connectivity between amygdala and medial prefrontal cortex: a dynamic functional connectivity and large-scale network perspective. *Front Syst Neurosci.* (2017) 11:29. doi: 10.3389/fnsys.2017.00029
129. Hou G, Yang X, Yuan TF. Hippocampal asymmetry: differences in structures and functions. *Neurochem Res.* (2013) 38:453–60. doi: 10.1007/s11064-012-0954-3
130. Bohbot VD, Iaria G, Petrides M. Hippocampal function and spatial memory: evidence from functional neuroimaging in healthy participants and performance of patients with medial temporal lobe resections. *Neuropsychology.* (2004) 18:418–25. doi: 10.1037/0894-4105.18.3.418
131. De Bellis MD, Hall J, Boring AM, Frustaci K, Moritz G. A pilot longitudinal study of hippocampal volumes in pediatric maltreatment-related posttraumatic stress disorder. *Biol Psychiatry.* (2001) 50:305–9. doi: 10.1016/S0006-3223(01)01105-2
132. Tupler LA, De Bellis MD. Segmented hippocampal volume in children and adolescents with posttraumatic stress disorder. *Biol Psychiatry.* (2006) 59:523–9. doi: 10.1016/j.biopsych.2005.08.007
133. Pederson CL, Maurer SH, Kaminski PL, Zander KA, Peters CM, Stokes-Crowe LA, et al. Hippocampal volume and memory performance in a community-based sample of women with posttraumatic stress disorder secondary to child abuse. *J Trauma Stress.* (2004) 17:37–40. doi: 10.1023/B:JOTS.0000014674.84517.46
134. Humphreys KL, King LS, Sacchet MD, Camacho MC, Colich NL, Ordaz SJ, et al. Evidence for a sensitive period in the effects of early life stress on hippocampal volume. *Dev Sci.* (2019) 22:e12775. doi: 10.1111/desc.12775
135. Rao H, Betancourt L, Giannetta JM, Brodsky NL, Korczykowski M, Avants BB, et al. Early parental care is important for hippocampal maturation: evidence from brain morphology in humans. *Neuroimage.* (2010) 49:1144–50. doi: 10.1016/j.neuroimage.2009.07.003
136. Roozendaal B, McEwen BS, Chattarji S. Stress, memory and the amygdala. *Nat Rev Neurosci.* (2009) 10:423–33. doi: 10.1038/nrn2651
137. Gamer M, Schmitz AK, Tittgemeyer M, Schilbach L. The human amygdala drives reflexive orienting towards facial features. *Curr Biol.* (2013) 23:R917–8. doi: 10.1016/j.cub.2013.09.008
138. Garrido MI, Barnes GR, Sahani M, Dolan RJ. Functional evidence for a dual route to amygdala. *Curr Biol.* (2012) 22:129–34. doi: 10.1016/j.cub.2011.11.056
139. Winstanley CA, Theobald DEH, Cardinal RN, Robbins TW. Contrasting roles of basolateral amygdala and orbitofrontal cortex in impulsive choice. *J Neurosci.* (2004) 24:4718–22. doi: 10.1523/JNEUROSCI.5606-03.2004
140. Pagliaccio D, Luby JL, Bogdan R, Agrawal A, Gaffrey MS, Belden AC, et al. Amygdala functional connectivity, HPA axis genetic variation, and life stress in children and relations to anxiety and emotion regulation. *J Abnorm Psychol.* (2015) 124:817–33. doi: 10.1037/abn0000094
141. Pechtel P, Lyons-Ruth K, Anderson CM, Teicher MH. Sensitive periods of amygdala development: the role of maltreatment in preadolescence. *Neuroimage.* (2014) 97:236–44. doi: 10.1016/j.neuroimage.2014.04.025
142. Bellis MD De, Keshavan MS, Clark DB, Casey BJ, Giedd JN, Boring AM, et al. Developmental traumatology part II: brain development. *Biol Psychiatry.* (1999) 45:1271–84. doi: 10.1016/S0006-3223(99)00045-1
143. Tyborowska A, Volman I, Niermann HCM, Pouwels JL, Smeekens S, Cillessen AHN, et al. Early-life and pubertal stress differentially modulate grey matter development in human adolescents. *Sci Rep.* (2018) 8:9201. doi: 10.1038/s41598-018-27439-5
144. Cohen RA, Grieve S, Hoth KF, Paul RH, Sweet L, Tate D, et al. Early life stress and morphometry of the adult anterior cingulate cortex and caudate nuclei. *Biol Psychiatry.* (2006) 59:975–82. doi: 10.1016/j.biopsych.2005.12.016
145. Rinne-Albers MA, Pannekoek JN, van Hoof MJ, van Lang ND, Lamers-Winkelman F, Rombouts SA, et al. Anterior cingulate cortex grey matter volume abnormalities in adolescents with PTSD after childhood sexual abuse. *Eur Neuropsychopharmacol.* (2017) 27:1163–71. doi: 10.1016/j.euroneuro.2017.08.432
146. De Bellis MD, Keshavan MS, Shifflett H, Iyengar S, Beers SR, Hall J, et al. Brain structures in pediatric maltreatment-related posttraumatic stress disorder: a sociodemographically matched study. *Biol Psychiatry.* (2002) 52:1066–78. doi: 10.1016/S0006-3223(02)01459-2
147. Teicher MH, Dumont NL, Ito Y, Vaituzis C, Giedd JN, Andersen SL. Childhood neglect is associated with reduced corpus callosum area. *Biol Psychiatry.* (2004) 56:80–5. doi: 10.1016/j.biopsych.2004.03.016
148. Teicher MH, Samson JA, Sheu YS, Polcari A, McGreenery CE. Hurtful words: association of exposure to peer verbal abuse with elevated psychiatric symptom scores and corpus callosum abnormalities. *Am J Psychiatry.* (2010) 167:1464–71. doi: 10.1176/appi.ajp.2010.10010030
149. Tomoda A, Navalta CP, Polcari A, Sadato N, Teicher MH. Childhood sexual abuse is associated with reduced gray matter volume in visual cortex of young women. *Biol Psychiatry.* (2009) 66:642–8. doi: 10.1016/j.biopsych.2009.04.021
150. Tomoda A, Polcari A, Anderson CM, Teicher MH. Reduced visual cortex gray matter volume and thickness in young adults who witnessed domestic violence during childhood. *PLoS ONE.* (2012) 7:e52528. doi: 10.1371/journal.pone.0052528
151. Heim CM, Mayberg HS, Mletzko T, Nemeroff CB, Pruessner JC. Decreased cortical representation of genital somatosensory field after childhood sexual abuse. *Am J Psychiatry.* (2013) 170:616–23. doi: 10.1176/appi.ajp.2013.12070950
152. Choi J, Jeong B, Rohan ML, Polcari AM, Teicher MH. Preliminary evidence for white matter tract abnormalities in young adults exposed to parental verbal abuse. *Biol Psychiatry.* (2009) 65:227–34. doi: 10.1016/j.biopsych.2008.06.022
153. Peng H, Ning Y, Zhang Y, Yang H, Zhang L, He Z, et al. White-matter density abnormalities in depressive patients with and without childhood neglect: a voxel-based morphometry (VBM) analysis. *Neurosci Lett.* (2013) 550:23–8. doi: 10.1016/j.neulet.2013.06.048
154. Choi J, Jeong B, Polcari A, Rohan ML, Teicher MH. Reduced fractional anisotropy in the visual limbic pathway of young adults witnessing domestic violence in childhood. *Neuroimage.* (2012) 59:1071–9. doi: 10.1016/j.neuroimage.2011.09.033

155. Wang L, Dai Z, Peng H, Tan L, Ding Y, He Z, et al. Overlapping and segregated resting-state functional connectivity in patients with major depressive disorder with and without childhood neglect. *Hum Brain Mapp.* (2014) 35:1154–66. doi: 10.1002/hbm.22241
156. Elton A, Tripathi SP, Mletzko T, Young J, Cisler JM, James GA, et al. Childhood maltreatment is associated with a sex-dependent functional reorganization of a brain inhibitory control network. *Hum Brain Mapp.* (2014) 35:1654–67. doi: 10.1002/hbm.22280
157. McLaughlin KA, Peverill M, Gold AL, Alves S, Sheridan MA. Child maltreatment and neural systems underlying emotion regulation. *J Am Acad Child Adolesc Psychiatry.* (2015) 54:753–62. doi: 10.1016/j.jaac.2015.06.010

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Feeding Habits in the Cultural Domains of Child Care: Elements for Health Promotion

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Introduction: Family eating behavior is determined by the meaning that the caretaker gives to food and the act of eating in the domestic environment, as well as the beliefs and perceptions around those concepts.

Objective: Identify the place that nutrition has within the dimensions of child care, the specific weight that the caregiver gives to it within the range of actions deployed and if there are differences when the child exhibits neurodevelopmental disorders, as a contribution to the design of interventions in health promotion.

Methodology: Qualitative, exploratory, two-stage study, with the approach of cognitive anthropology; proposal sampling of maximum differences, 121 informants participated in three groups, caregivers of: (1) healthy children, (2) children who had been hospitalized between 3 and 6 months prior to the time of the interview, and (3) children with a diagnosis of permanent neurological injury and that express some type of neurodevelopmental disorder.

Results: Nourishment is the element that reaches the highest values of cultural relevance in the three groups, is located in different domains according to the condition of the care receiver.

Conclusion: The common domains are Well-being, Health Maintenance, Coexistence, and Security, in the 3rd group the domain of Socialization emerges, the elements that make up the conceptual dimensions were identified, the comparative design allowed to identify differences. The description of the domains can represent the cognitive spaces of educational intervention and the elements that configure them are the triggers of the interaction, due to the importance they are given in everyday life.

Keywords: infant feeding, qualitative research, child care, health promotion, cultural domains

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INTRODUCTION

The usual care that a young child receives includes actions that are aimed at guaranteeing survival, growth, and well-being. Nutrition, as an essential process for life, is a constitutive element of child care. There are multiple factors that intervene so that the diet that an infant receives meets her nutritional needs. Various studies have shown that socioeconomic conditions are strongly related to the ability to acquire food. It has also been observed that the existence of parental and social networks represent a support to mitigate precarious conditions and favor the nutritional conditions of groups with social and economic disadvantage (1, 2).

On the other hand, within the framework of the normative values of their environment, subjects generate cultural decisions and carry out actions in accordance with the information they have acquired and the way they have processed it. The meaning, beliefs and perceptions that the caregiver has of food and the act of eating in the family environment are related to the eating behavior of the family. It is important to highlight that from Anthropology common elements have been identified in the eating patterns, both of populations that inhabit different geographic contexts (3) and in others, with diverse historical antecedents, which is related to one of the most important characteristics that human beings have, their adaptive capacity to the natural and social conditions of the environment (4, 5).

The family environment is the scenario in which the caregiver decides the actions related to feeding, as a complex phenomenon, and thus, turns them into a stimulus and result of the integral child development process, in this way, in everyday life, she expresses, what Paris Aguilar mentions as "the capacity of the artisan production of facts and meanings" (6, 7).

Bronfenbrenner's theoretical approach (8, 9), referring to human development, is appropriate to understand the importance of food and the context in which it is carried out, both in its nutritional aspect and in its purpose of favoring the socialization of the child; processes carried out within the family and in which particularly the mother, constitutes an ideal mediating factor between the family nucleus and the school and social group. The close interaction with the mother in her immediate environment and the emotional attachment established between the mother-child dyad are elements that positively trigger the interconnection of the child's family microsystem with more complex environments, represented by the mesosystem, exosystem and macrosystem, structures social and cultural conditions that enable appropriate child development, and it is precisely the moment when the child is fed when the interaction, approach and communication of the mother-child dyad are closer.

In Mexico, food policies and programs have been designed that have allowed these highly complex structures, particularly health and education institutions, to interact favorably with the elements that are located in the immediate environment of school children and their mothers, with the purpose of maintaining health and promoting well-being. During the last 100 years, the actions of the food programs have been aimed at three fundamental aspects: (a) Support for food production, (b) Diversification and strengthening of the food collection, storage, and distribution network, and (c) Provision of subsidies and direct interventions to vulnerable groups to modify food consumption patterns, with a weak presence of educational interventions (10).

Despite the wealth of knowledge accumulated around the topic of infant nutrition, it contrasts with the difficult penetration observed in health promotion programs aimed at strengthening family eating practices, either to preserve favorable behaviors, or to modify or avoid negative behaviors. The approaches carried out about food and nutrition, both biomedical and epidemiological, which were pioneers in Mexico, were oriented more to the knowledge of environmental conditions, economic, and sociodemographic characteristics of individuals, than to the investigation of the aspects relevant social and cultural characteristics of the investigated communities (11, 12).

Although the relevance of these investigative approaches is indisputable, the information that would allow the design of health promotion programs was not used. In reality, the development of such programs has been based on the knowledge generated from the perspective of nutrition experts, that is, considering the dietary norm more than the social or cultural norm, so it is urgent to recover the look and testimony of those who care for and feed children, according to their beliefs and meanings (13, 14).

The interactions of social life are manifested in the small daily phenomena that progressively constitute the social fabric and that once all this has settled down can emerge a deeper knowledge closer to reality. This knowledge or familiarity is what Heidegger calls the world. The world is taken for granted and seen and is only noticed in situations of rupture; the worlds in which people live are not universal or timeless, on the contrary, they are different according to the culture, time or historical period and according to the family in which they are born. Qualitative distinctions give meaning to things recognized by the person in their daily life and are shaped by culture and language (15). Therefore, if health promotion programs are designed without taking into account the components of the cultural concept of food, without elucidating the reasons that underlie food preparation decisions, the health promotion programs will continue to advance in parallel lines promoting the health and behaviors of the population.

The model provided by the Cultural Consensus Theory explores how subjects generate cultural decisions and perform actions according to the information they have acquired and the way they have processed it, within their normative values. It allows this by recognizing the terms that the informants express as ideas or actions, the relevance that these terms have in the

TABLE 1 | Characteristics of the participating groups.

	No. of participants	Child age (mean)	Caregivers age (mean)	Reason by gender male/female
Group A				
Healthy Child Caregivers	38	24 months	27 years	1/11.6
Group B				
Caregivers of hospitalized children in the past three to six months	40	24 months	28 years	1/9
Group C				
Caregivers of children with permanent neurological injury	43	37 months	37 years	1/7.6
Total	121			

majority of the members of the group and the grouping pattern that is observed when looking for similarities between them, always from the point of view of the informants (16).

Weller and Romney define the cultural domain as a group of phrases or words linked by semantic relationships, which together refer to a specific conceptual sphere; they represent perceptions more than preferences; they derive their meaning, in part, from their position in a mutually interdependent system, which reflects the way in which a cultural group classifies the relevant conceptual sphere. It is possible to explore two aspects of the normative beliefs of the group: The qualitative aspect, through the grouping pattern of individual responses, while the quantitative aspect refers to the relevance attributed to each response as a result of the frequency of the response hierarchical.

There is consensus in the conceptual sphere when there is correspondence between the expressions of each informant with those of the rest of the group. Those informants who show greater correspondence with the group are classified as competent informants, due to the possibility that they contribute diverse and sufficient experiences as shared knowledge of the phenomenon under study.

To use the formal Cultural Consensus Model in the analysis of the information obtained by free lists and pile sort, it is

TABLE 2 | Elements of greater cultural relevance, conformation of the domains by pile sort.

Cultural relevance		Domain	Cultural relevance		Domain	Cultural relevance		Domain
1	Feeding	2	1	Feeding	1	1	Personal care	2
2	Avoid accidents	5	2	Homeclean	3	2	Feeding	4
3	Take care	3	3	Child cleaning	3	3	Cleaning	2
4	Loving	3	4	Watch out	2	4	Avoid accidents	3
5	Homeclean	2	5	Avoid accidents	1	5	Be aware	2
6	To be alert	2	6	Watch what he eats	1	6	Hygiene	2
7	Watch over	5	7	To play	2	7	Give love	4
8	Cleanliness	2	8	Clean toys	3	8	Don't approach fire	3
9	Bath	2	9	Coexistence	2	9	Correct behavior	4
10	To play	3	10	Attention	2	10	Family life	4
11	Coexistence	5	11	Loving mother	2	11	Attend needs	1
12	Education	2	12	Give medicine	1	12	Concer	1
13	Avoid disease	1	13	Go to the doctor	1	13	No things to mouth	3
14	Therapy	4	14	Communication	2	14	To play	4
15	Go to the doctor	1	15	Necessary medicine	1	15	Watch out	2
16	Give medicine	1	16	Hygiene	3	16	Fatigue	1
17	Stimulation	4	17	Enough sleep	1	17	Go to the therapy	1
18	Follow	4	18	Patience	2	18	Don't approach plugs	3
19	Patience	3	19	Love	2	19	Don't leave home	3
20	Deceive	5	20	No pets	3	20	Education	4

TABLE 3 | Cultural relevance of food and Consensus of cultural domains of "child care," according to group of caregivers.

Group	Cultural relevance (CR)		Cultural consensus		
	Place of the feeding between the 20 elements	Value of CR	Factor 1 (F1)* and % of cumulative variance	Factor 2 (F2) and % of cumulative variance	Ratio F1/F2**
1	First	0.238	6.709	1.827	
$n = 38$					
			81.3%	93.4%	3.67
2	First	0.355	8.207	2.034	
$n = 40$					
			84.6%	94.9%	4.03
3	Second	0.200	9.153	2.018	
$n = 43$					
			86.0%	95.3%	4.53

Durango, Dgo., Mexico.

**Cumulative variance of F1 > 60% = It is homogeneous.*

***Ratio F1/F2 > 3 = There is consensus.*

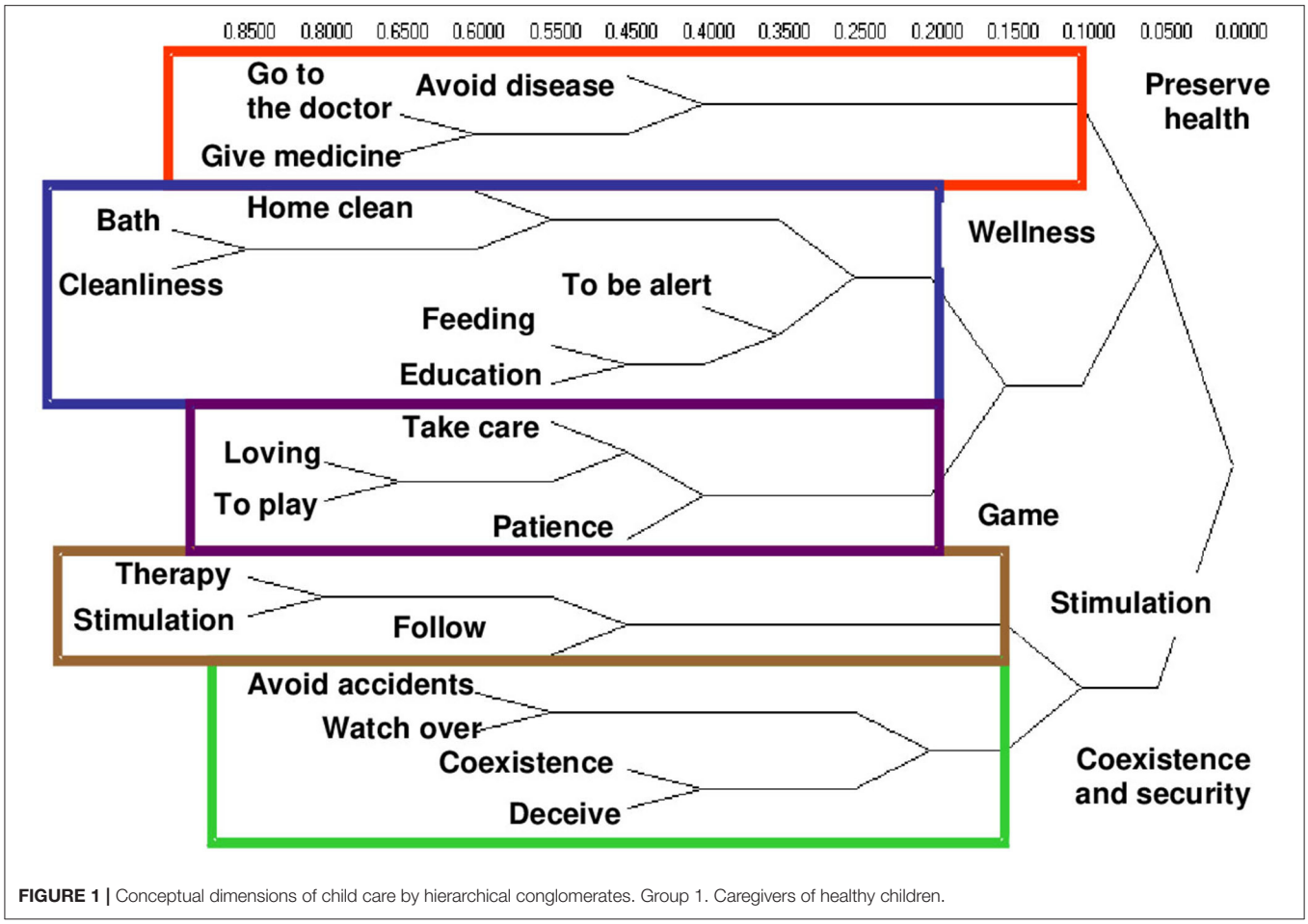

FIGURE 1 | Conceptual dimensions of child care by hierarchical conglomerates. Group 1. Caregivers of healthy children.

necessary that the answers be words or short phrases, they are answered individually, they are oriented to the same topic with the same level of complexity, and respect the "fresh" answer, that is, without correction or transformation (17). The study of cultural domains has been used successfully before various health problems (18–20).

In order to identify the location of food within the cultural domains of child care, according to the health condition of the care recipient, a two-stage, phenomenological qualitative study was designed within the framework of the cultural consensus theory.

General Objective

Identify the site and the relevance given to food within the cultural dimensions of child care.

Specific Objective

Know the differences in the configuration of cultural domains when the care recipient suffers temporary or permanent illness.

Design

Qualitative exploratory study, in resident population of the city of Durango, Dgo., Mexico.

Informants

Three groups of preschool child caregivers were formed by propositive sampling and maximum differences:

Group 1—38 healthy child caregivers,

Group 2—40 caregivers of children who had been hospitalized between 3 and 6 months prior to the time of the interview, and

Group 3—43 caregivers of children diagnosed with permanent neurological injury and who expressed some type of neurodevelopmental disorder.

In determining the number of participants per group, the assumption of the consensus model was taken as the main criterion, which suggests for studies of cultural description a minimum size of 17 informants to classify 95% of the questions correctly, because the correlation Average among informants tends to be high. The participants of each group were divided equally for the realization of the two stages of the work.

Operational Stages

The meeting scenarios for the collection of information were the pediatric service of the General Hospital of Durango, Dgo and the Integral Development Care Center "Dr. Isauro Venzor (CADI) of Durango. One hundred and twenty one semi-structured interviews were conducted, fifty-eight and sixty-three respectively, free listings were obtained. The response rate was 100%.

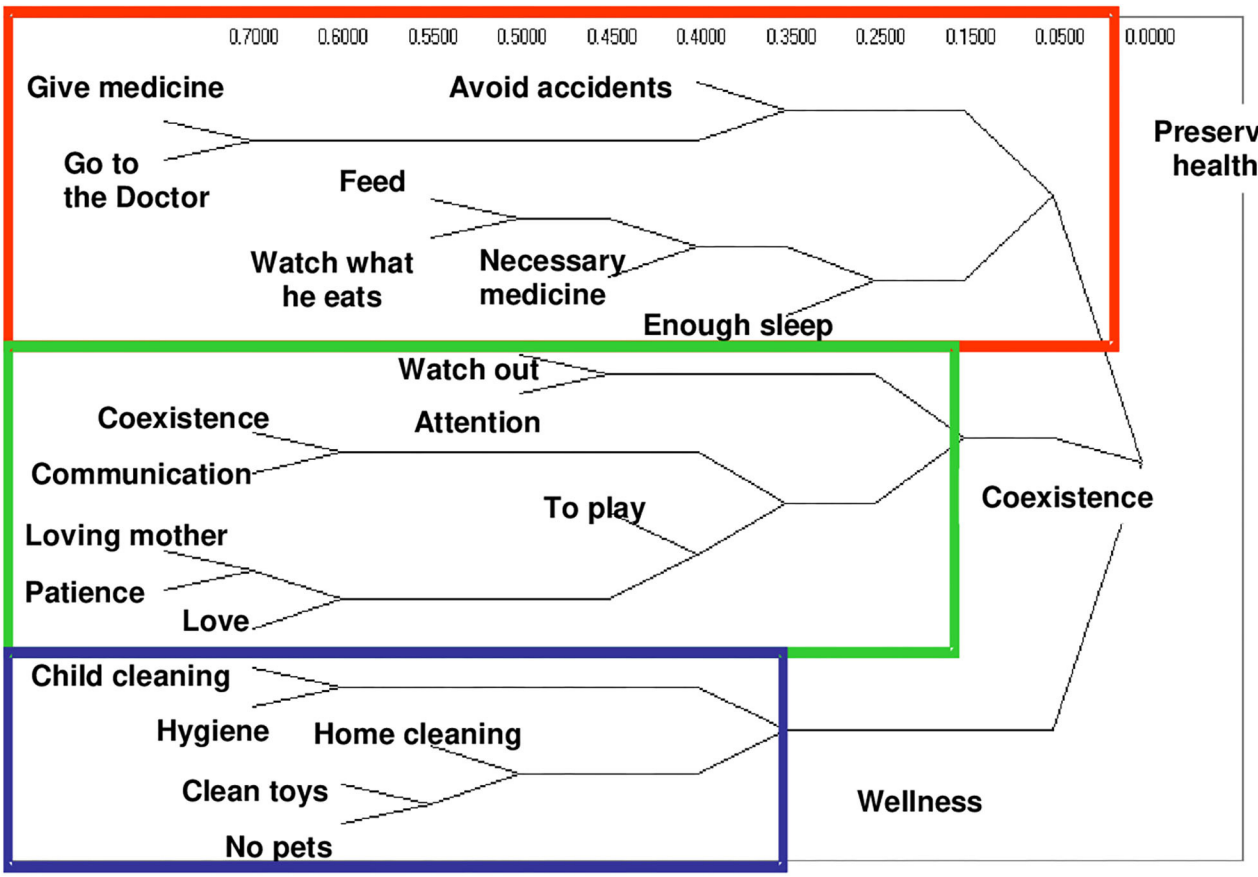

FIGURE 2 | Conceptual dimensions of child care by hierarchical conglomerates. Group 2. Caregivers of children hospitalized 6 months ago.

The information was analyzed with support of Anthropac 4.1 and Ucinet software.

Stage 1. Obtaining free listings. They were prepared by registering the first 10 short sentences or words that the informant mentioned when asked what she was looking for child care; once the free listings were collected and in a tabular form the particular elements were identified, the absolute and relative frequency with which each of these elements is mentioned and the place in which they appeared in the lists, the relevance index was determined cultural or smith index, which is the product of the relationship between the previous values, that score represents the specific weight that each element has as part of the concept of care, however, it does not allow to discover the relationship that each of these elements have with each other, nor the way in which are configured the cultural dimensions, for that the next stage is necessary.

Stage 2. Drawing lots. The 20 elements that reached the highest figures of the cultural relevance index (smith index) were selected and printed on cards; the cards were assigned the numerical code corresponding to the cultural relevance index, so that the components of each group could be identified and individual values assigned. The participants gathered the cards that had the terms that looked the most and formed 4–5 lots each.

Individual values were analyzed using a factorial model that estimates the likelihood that a homogeneous system of knowledge predominates in a culture, based on the correspondence between the knowledge of the group and those of the individual. The likelihood criterion for obtaining consensus in the models implies that the first factor reaches a ratio three times greater than that of the second factor, condition that is interpreted as concordance in the responses of the informants, it is also possible to identify the similarity and the difference between the dimensions that make up the cultural domains of each group (21, 22). The aggregate values were analyzed by hierarchical conglomerates, to identify the degree of perceived similarity among the participants and is represented in a tree diagram with correlation levels.

RESULTS

The primary caregiver is the mother in the caregiver groups of the three groups of children, although the participation of the father is more frequent in the group of children with developmental disorders than in the other two groups of children (**Table 1**). Nourishment is the element that reaches the highest values of cultural relevance in the three groups of caregivers, it is

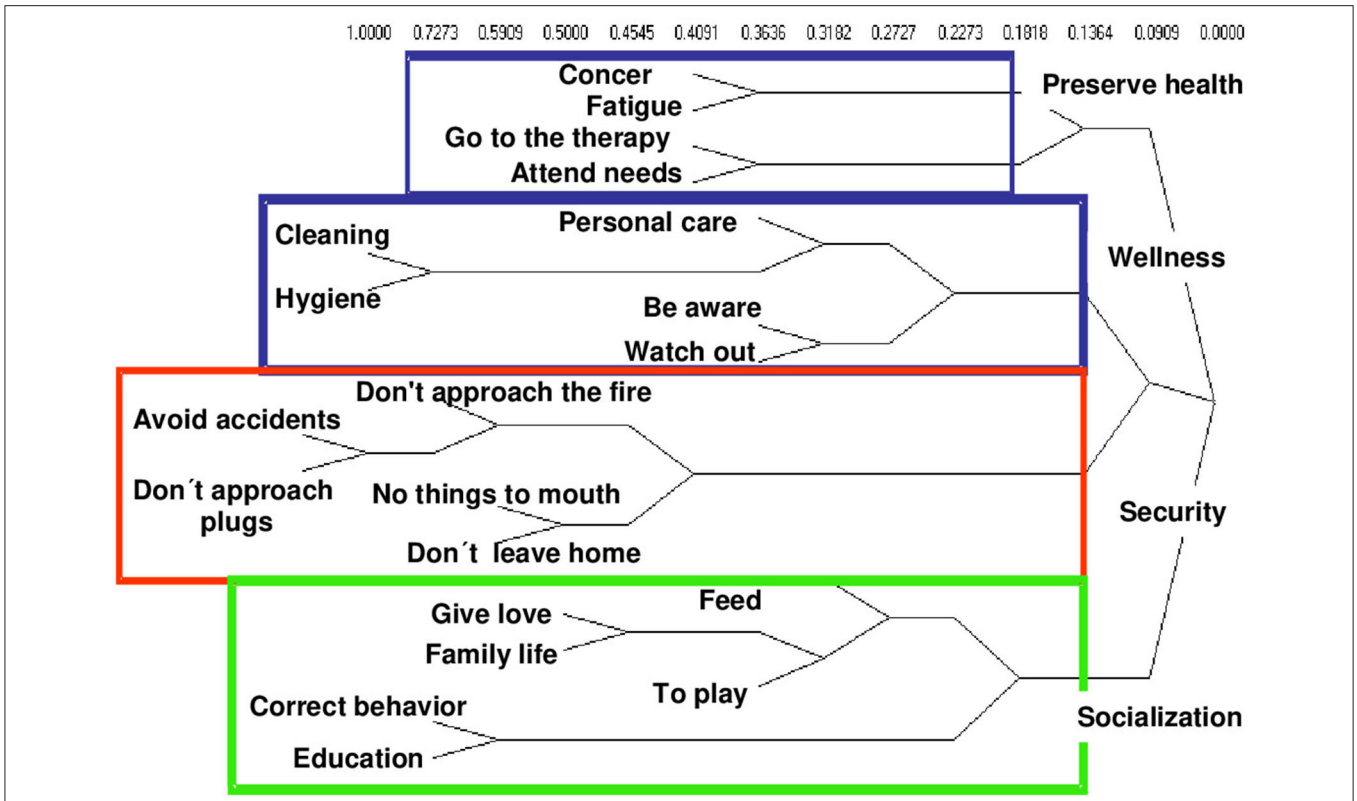

FIGURE 3 | Conceptual dimensions of child care by hierarchical conglomerates. Group 3. Caregivers of children with developmental disorders.

appreciated that it occupies the first and second place among the first 20 elements (**Table 2**).

The conceptual dimensions of "child care" show homogeneity, the percentage of accumulated variance for the first factor is 81.3, 84.6, and 86.0, respectively, when it is >60%, it is homogeneous—and consensus is observed in the three groups, the ratio between the first and second factor is 3.67, 4.03, and 4.53—when it is >3, consensus is considered to exist (**Table 3**).

The dimensions that appear in the three groups show similarities because of the presence of the particular elements, however, when observing the arrangement and the relationship between them, the dimensions are configured differently.

In group 1, made up of the mothers of healthy children, food is part of the welfare dimension, which includes body hygiene, bathing and household cleaning, among others (**Figure 1**).

In group 2, that of the caregivers of children who have had a period of hospitalization between 3 and 6 months prior to the time of the interview, feeding is located in the dimension of health conservation what is accompanied by the care in what they eat and also in buying the necessary medicines (**Figure 2**). While in group 3, caregivers of children with neurodevelopmental disorders place food in the socialization dimension, in which they share space with actions aimed at avoiding social isolation, and favors the incorporation of child to the family environment, the neighborhood, and the school (**Figure 3**).

DISCUSSION

The use of an anthropological approach in the study of the feeding of preschool children with different health conditions, allows to have an appropriate cultural and social perspective, to understand the impact that the caregivers of these children have, in the care they grant on the nutritional and relational level, particularly when it comes to preschoolers with neurological damage.

Infant feeding is the field in which the mother performs one of the activities that represents a relevant dimension of parenting and that she perceives as a significant feature of "being a mother," which involves participating and deciding on the child's development. It is important to mention that it is also a space for participation and decision of the baby about its immediate environment. It is a time of intense interaction where the needs of the baby and the care of the mother are faced.

The emotional symbolic universes make possible the cultural patterns and norms that modulate both the form of language acquisition and the behavior patterns that allow communication, the exchange between the subjects, and the reproduction and transformation of the social order (23).

The group of caregivers of children with permanent injuries identify feeding on the same level as "play, give love, family life, correct behavior, and education," so that a nuance of socialization is appreciated; unlike what the other groups refer to, this is part

of the dimension in which recreational, affective activities that promote discipline and education are incorporated; they give great cultural relevance to alimentation for different reasons than the caregivers of the other two groups, these caregivers have more experience in the home care process, they seem to have more complex cultural constructions on this phenomenon, they have turned feeding into a space for interaction, they consider it as the opportunity to modify or adapt the behaviors linked to maternage, particularly related to the conditions of the baby's social performance, of the mother and the rest of the family, allows them to identify problems in the relationship established by the child with those responsible for their care, it could be that they have left behind hospital crises, family crises and the grief of not having had a healthy child; the caregivers are located in another plane, which becomes more understandable from the theoretical perspective of Bronfenbrenner and Morris (24), who defines human development as an enduring change in the way in which the person perceives the environment and relates to it. Although the Bronfenbrenner model has the child as the central protagonist, the importance of the caregiver's role is unquestionable, as a character that is responsible for "creating" the environments and "building" relationships, which allow the child to be optimally incorporated into its environment; if this is important when it comes to a healthy child, in the case of children with impaired development, it is crucial. To understand the feeding habits from a cultural perspective, it is necessary to design methodological approaches that allow access to symbolic universes and the study of social processes that are articulated with their properties and meanings, particularly the internal meanings of alimentation (3).

CONCLUSION

The elements that make up the conceptual dimensions of child care were identified, as well as the prominent role given to food. The comparative design allowed to identify substantive differences in the perception that the caregiver has about what feeding means. When the child is healthy, she is fed to maintain well-being. If the child becomes ill, food becomes a necessary element to regain health and avoid illness, while when the child is faced with a developmental alteration as a permanent condition, the physical and temporary space in which the food, is used to promote social interaction. The meaning that the caregiver gives to food can be the starting point in the design of health promotion programs.

If the caregiver received guidance that allowed her to reinforce the perception of the usefulness of the diet, a bond of trust would be created with the health personnel, due to the consonance of the approaches and from there, the reception of the messages could be facilitated and practices aimed at establishing healthy eating habits.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/supplementary material.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by this work is part of a Doctoral Thesis. The ethical aspects of the project were reviewed and approved by the Doctoral Program Tutorial Committee of the University of Guadalajara, Mexico. Participating mothers were informed and invited to participate in the study. They expressed their acceptance and signed an informed consent letter. The research consisted of conducting interviews with the participating mothers. The questionnaires are anonymous and confidential. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

The contributions of YM-L, JS-M, and NA-A were similar in theoretical, method, and results analysis aspects. YM-L wrote the article. All authors contributed to the revision of the manuscript, read it, discussed, and approved the final version.

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REFERENCES

1. Sandra Lucía Restrepo M, Maya Gallego, M. La familia y su papel en la formación de los hábitos alimentarios en el escolar. Un acercamiento a la cotidianidad. *Bol Antropol.* (2005) 19:127–48.
2. Barker M, Lawrence WT, Skinner TC, Haslam CO, Robinson SM, Inskip HM, et al. Constraints on food choices of women in the UK with lower educational attainment. *Public Health Nutr.* (2008) 11:1229–37. doi: 10.1017/S13689800080 0178X
3. Bertran M. Significados socioculturales de la alimentación en la Ciudad de México. In: Bertran M, Arroyo P, editors. Antropología y Nutrición, Fondo Nestlé para la Nutrición. Funsalud. México: Universidad Autónoma Metropolitana -Xochimilco (2006). p. 221–35.
4. Pérez S, De los Ángeles M. *History of the food's History.* Chron Nova (2009) 35:105–62.
5. Fischler, C. El (h)omnívoro. *El gusto, la cocina y el cuerpo.* Barcelona: Anagrama (1995). p. 28–87.
6. Moreno AC, Carbajal MMM, Larios NG, Villarreal LI, Addalid SH. Crianza, nutrición y desarrollo infantil en niños de Sonora y

del Estado de México. *Rev Mex Trastor Aliment.* (2018) 9:170–82. doi: 10.22201/fesi.20071523e.2018.2.480
7. Piña PA. Cultura y alimentación. *Aspectos fundamentales para una visión comprensiva de la alimentación humana Anal Antrop.* (2014) 48:11–31. doi: 10.1016/S0185-1225(14)70487-4
8. Bronfenbrenner U, Morris PA. The bioecological model of human development. In: Lerner RM, Damon W, editors. *Handbook of Child Psychology: Theoretical Models of Human Development*. John Wiley & Sons Inc (2006). p. 793–828. doi: 10.1002/9780470147658.chpsy0114
9. Vélez-Agosto NM, Soto-Crespo JG, Vizcarrondo-Oppenheimer M, Vega-Molina S, Coll CG. Bronfenbrenner's bioecological theory revision: moving culture from the macro into the micro. *Perspect Psychol Sci.* (2017) 12:900–10. doi: 10.1177/1745691617704397
10. Barquera S, Rivera-Dommarco J, Gasca-García A. Food and nutrition policies and programs in Mexico. *Salud Publ Mex.* (2001) 43:464–77. doi: 10.1590/S0036-36342001000500011
11. Aguirre-Arenas J, Escobar-Pérez M, Chávez-Villasana A. Evaluación de los patrones alimentarios y la nutrición en cuatro comunidades rurales. *Salud Públ Méx.* (1998) 40:398–407. doi: 10.1590/S0036-36341998000500003
12. Saucedo Arteaga G, Chávez Villasana A, Merino Zeferino B, Madrigal Fritsch H. Cambios en el déficit de talla de los preescolares en la población rural mexicana. *Estud Antropol Biol.* (2001) 10:159–72.
13. Pérez Gil S. Percepciones y prácticas alimentarias de un grupo de mujeres en el México rural. In: *Bertran M, Arroyo P, editors. Antropología y Nutrición.* México: Fondo Nestlé para la Nutrición. Funsalud; Universidad Autónoma Metropolitana -Xochimilco (2006). p. 137–55.
14. Contreras J, Gracia Arnaiz M. Del dicho al hecho: las diferencias entre las normas y las prácticas alimentarias. In: *Antropología y Nutrición. México: Fondo Nestlé para la Nutrición*. Funsalud; Universidad Autónoma Metropolitana -Xochimilco (2006). p. 75–115.
15. Esguerra Lozada MM. The world in the Heidegger's Phenomenology. An approach to the world from the History of the Concept of Time: Prolegomena. *Praxis Filosófica.* (2018) 46:151–69. doi: 10.25100/pfilosofica.v0i46.6165
16. Weller SC. Cultural consensus theory: applications and frequently asked questions. *Field Methods.* (2007) 19:339–68. doi: 10.1177/1525822X07303502
17. Weller SC, Romney K. *Systematic Data Collection.* Newbury Park, CA: Sage (1988).
18. Hoyos RC, López TT, Villarreal FC, Lucatero AP, González MA, Coutiño BL. Concepciones culturales sobre el dengue en contextos urbanos de México. *Rev Saúde Públ.* (2006) 40:126–33. doi: 10.1590/S0034-89102006000100020
19. Aguilera V, de los Ángeles M, López TMT, Rodríguez García RM, Acosta Fernández M. Dimensiones culturales del concepto de salud en trabajadores jubilados de guadalajara, México. *Hacia Prom Salud.* (2010) 15:13–27.
20. Kalra N, Pelto G, Tawiah C, Zobrist S, Milani P, Manu G, et al. Patterns of cultural consensus and intracultural diversity in Ghanaian complementary feeding practices. *Matern Child Nutr.* (2017) 14:e12445. doi: 10.1111/mcn.12445
21. Sturrock K, Rocha JM. A multidimensional scaling stress evaluation table. *Field Methods.* (2000) 12:49–60. doi: 10.1177/1525822X0001200104
22. Weller S. Assesing rater performance without a gold standard using consensus theory. *Med Decis Mak.* (1997) 17:71–9. doi: 10.1177/0272989X9701700108
23. Calderón E. El papel de la dimensión afectiva en la adquisición del lenguaje materno. *Alteridades.* (2017) 27:11–22.
24. Bronfenbrenner U, Morris PA. The ecology of cognitive developmental processes. In: Damon W, Lerner RM, editors. *Handbook of Child Psychology. Vol. 1. Theoretical Models of Human Development*. New York, NY: John Wiley & Sons, Inc. (1998). p. 993–1028.

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ORIGINAL RESEARCH
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Effect of Bacterial Vaginosis (BV)-HIV-1 Co-existence on Maternal and Infant Health: A Secondary Data Analysis

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Background: The lactobacillus-rich microbiome forms a defense system against infections. Babies are born sterile and acquire their microbiome from exposure to the mothers' vaginal and rectal microbiota. Bacterial vaginosis (BV), which is characterized by a deficit of the Lactobacilli genera, may predispose women and their babies to an increased frequency of illness.

Objective: To determine the effect of BV on HIV-infected women's post-delivery health as well as the morbidity and mortality of the exposed infant at birth, 6 months, and at 12 months of life.

Study Design: A retrospective cohort study was conducted using previously collected data to investigate whether there was an association between BV-HIV-1 infected mothers and subsequent infant morbidity and mortality over a 12-month period.

Methods: Data for this analysis were extracted from the original data set. Women were categorized into two groups according to whether they had a positive or negative laboratory-based diagnosis of BV using the Nugent method. The two groups were compared for socio-demographic characteristics, prior to the pregnancy experience in their current pregnancy outcome and at post-delivery morbidity, and for the duration of hospital stay. BV-exposed and unexposed infants were compared in terms of morbidity and mortality at birth, and in the periods between birth and 6 months, and between 6 and 12 months, respectively, based on prospectively collected data of the mother's past and present illness, and clinical examination at scheduled and unscheduled visits during the follow-up period of the original study. The generalized estimating equation (GEE) was used to analyze the longitudinally collected data. We used the Kaplan-Meier (KM) method to generate the cumulative hazard curve and compared the mortality in the first year of life between the two groups.

Results: In total, 365 patients were included in the study. Exposure to BV was associated with an adverse maternal condition (Relative Risk [RR], 2.45; 95% confidence interval [CI], 1.04–5.81, $P = 0.04$) and maternal hospital admission (RR, 1.99; 95% CI, 1.14–3.48, $P = 0.02$) but was not linked to any neonatal morbidity at birth. There was a higher frequency of gastro-intestinal morbidity among BV-exposed infants. At 6 months,

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infants of BV-exposed mothers had higher odds of bloody stool (Odds Ratio [OR], 3.08; 95% CI, 1.11–10.00, $P = 0.04$), dehydration (OR, 2.94; 95% CI, 1.44–6.37, $P = 0.01$), vomiting (OR, 1.64; 95% CI, 1.06–2.56, $P = 0.03$), and mouth ulcers (OR, 12.8; 95% CI, 2.27–241.21, $P = 0.02$). At 12 months, exposure to BV was associated with dehydration (OR, 1.81; 95% CI, 1.05–3.19, $P = 0.03$) and vomiting (OR, 1.39; 95% CI, 1.01–1.92, $P = 0.04$). KM survival analysis showed non-significant higher trends of deaths among BV-exposed infants ($P = 0.65$).

Conclusion: This study demonstrates differences in maternal and infant morbidity outcomes associated with exposure to BV. Further research is required to determine whether treatment for maternal BV mitigates maternal and infant morbidity.

Keywords: mortality, HIV, infant, bacterial vaginosis, morbidity

1. INTRODUCTION

Bacterial vaginosis (BV), also referred to as vaginal dysbiosis, is characterized by altered vaginal biota, which typically has a deficit of *Lactobacillus bifidus* and an overgrowth of anaerobic polybacteria that include *Clostridiales, Fusobacterium nucleatum, Gardnerella vaginalis, Mycoplasma hominis,* and *Bacteroides urealyticus*, among others. BV may co-exist with other anerobic bacteria, such as *Streptococcus, Staphylococcus*, and *Enterobacteriaceae*, typically found in the lower genital tract (1).

BV has been shown to be a risk factor for pre-term births (2). A pre-term birth is defined as a delivery before 37 completed weeks of gestation either from labor induced due to a fetal condition, from spontaneous labor with intact membranes, or following a premature rupture of membranes (PROM). The latter is more common among Black women and is attributed to genital tract infection in both human immunodeficiency virus (HIV) seronegative and seropositive women (2–4). Prematurity in the presence of BV is additionally associated with a low birth weight (LBW) (5, 6). It is estimated that annually, about 13 million babies are born before 37 completed weeks of gestation; BV-related deaths account for 75% of the total perinatal deaths and 27% of the total neonatal mortality cases. This makes BV a condition of significant public health importance and requires further evaluation (2, 3, 7, 8).

Infant-related adverse effects of BV exposure are not limited to causing pre-term births, as illustrated in a retrospective review of 12,340 mother–baby pairs identified from Washington State medical records, which found that BV-exposed babies were more likely to have meconium staining at birth, which is indicative of *in-utero* stress. In the same study, independent of meconium staining and chorioamnionitis, BV-exposed term babies were at an increased risk of respiratory distress requiring ventilatory support, admission to a neonatal intensive care unit (NICU), and neonatal sepsis (9). There are limited data on whether BV has an effect on infant morbidity and mortality beyond the immediate newborn period.

A healthy vaginal microbiome has a preponderance of the Lactobacillus genera (2). These anaerobic bacteria contribute to an acidic environment by fermenting sugars that produce lactic acid. The acidic environment helps protect the uterus from ascending infections, such as sexually transmitted diseases and urinary tract infections. The same principle applies for other body surfaces including the gastrointestinal system. There is enough evidence showing that good bacteria in women lower the risk of infections (10) and HIV (11). Although there are no known direct causes of BV, a large body of literature reports an increased probability of infection due to sexual activity. Two studies conducted in Kenya provide evidence for this with the observation that 90% of sexually inactive women had normal vaginal flora (12, 13), whereas Bukusi et al., reported a high BV prevalence of 44% among married couples (14). BV, however, does not meet the threshold of being classified as a sexually transmitted infection because the male partner does not experience any illness and there is lack of a single causative agent.

BV often co-exists with sexually transmitted infections. A meta-analysis of 37,000 HIV-negative women from sub-Saharan Africa found that >50% of women with different STDs had co-existing BV and there was a significant correlation between BV and herpes simplex type 2 (HSV-2) and Trichomonas (15). BV increases women's risk of acquiring HIV (16, 17), and once infected, it is associated with the increased genital shedding of HIV, a risk factor for intrapartum HIV transmission (18). BV-HIV co-infection is associated with a 3-fold increased risk of *in-utero* mother-to-child transmission of HIV after adjusting for maternal HIV-1 viral load (19).

African women carry a disproportionate burden of BV. In the meta-analysis published by Torrone et al., the prevalence of BV among HIV-negative women in sub-Saharan Africa was >40% higher in the high-risk groups, compared to women recruited at community level or at health clinics, but the difference was not significant (49.5 vs. 35.2%) (15). An equally high prevalence of BV has been described among HIV-infected women; 46% in a high-risk North American population (20) and 47% in this group of Kenyan women who are the subject of this secondary data analysis (21).

At birth, infants are sterile, and current birthing practices are designed to optimize newborn exposure to maternal biota for which there is already immunologic protection through transplacental immunoglobulin transfer and through breastfeeding, thereby reducing the risk of infection, which is

one of the leading causes of newborn deaths (7, 22). There is evidence that a newborn's gut biota is derived from the mother. Breastmilk has the bifidus factor, a product that promotes the growth of lactobacilli in the infant gut. Since BV is a state of vaginal dysbiosis characterized by a deficit in *L. genera* that form an important defense mechanism in the infant gut and other mucous membranes, we hypothesized that it may be correlated with an increase in infectious disease morbidity beyond the newborn period.

HIV-exposed infants have increased vulnerability. In the first instance, BV increases the risk of maternal HIV acquisition (16, 17). Furthermore, HIV-infected women have reduced maternal transfer of immunoglobulins against common infections to the fetus (22). This study provides an opportunity to determine whether exposure to BV further accentuates the vulnerability of HIV-exposed infants.

There is emerging evidence that child survival is closely linked to a mother's vital status; the younger a child is when a mother dies, the more likely it is that the child will die (23). Nguyen et al. documented a 35-fold higher risk of infant death in the first 6 months of life, if the mother's death was before 42 days of life (23). In the present study population, we previously reported that there was a nearly 8-fold increased risk of death when the mother died (21). The present study aimed to investigate whether there are any differences in the health of mothers and infants exposed to BV after birth. This study is a secondary analysis based on a 25-year-old dataset of the randomized trial of breastfeeding and formula-feeding among HIV-infected women, carried out to determine the risk, timing, and correlation of transmission before the availability of anti-retroviral therapy by Nduati et al. The study population is unique in the sense that the women were well-investigated for sexually transmitted infection during pregnancy, and there was a careful structured longitudinal follow-up of the babies up to 24 months of life, which has enabled the research team to carry out various analyses based on this data set. The findings of this study contribute to a better understanding of the role of BV in maternal and child health and informs policies regarding the same.

2. METHODS

2.1. Study Design

This analysis is based on a retrospective cohort study design. The study methods that were used for the original study have previously been described (21). Briefly, the study was conducted in Nairobi with active enrollment from November 6, 1992 to October 7, 1997.

2.2. Study Population, Enrollment, Delivery, and Follow-Up

2.2.1. Study Population

The original study recruited 425 women, 401 of whom had live births. This study is restricted to 365 pairs who had complete matching information in terms of the BV status of the mother and requisite neonatal characteristics and included 180 women with BV and their exposed infants and 185 without BV. A total of 36 mother baby pairs were excluded because of missing information on the BV status as depicted by the flowchart shown in **Figure 1**. The analysis of the infant data was scaled down to 328, including 157 and 171 infants who were BV-exposed and unexposed, respectively. Thirty-seven pairs were excluded from further analysis for the following reasons: 14 babies died, and their morbidity measures were not assessed thereafter, 6 mothers were lost to follow-up, and 17 had missing data. These were finally included in the generalized estimating equation (GEE) analysis.

2.2.2. Maternal Enrollment

Pregnant women attending Nairobi City Council clinics were offered HIV testing as an integrated pregnancy assessment and those who tested positive were offered participation in the study. At 32 weeks of pregnancy, after obtaining informed consent, women were subjected to a standard interview and physical examination that included a pelvic examination using a speculum to facilitate collection of vaginal and cervical secretions for microscopy and gram staining for BV, in addition to screening for sexually transmitted diseases and genital shedding of HIV. Cervical and vaginal samples were collected separately using sterile Dacron swabs. To determine the viral load and CD4—8 cell counts, 15 ml of blood was collected in purple-top vacutainers at enrollment.

2.2.3. Delivery

Women were encouraged to deliver at Kenyatta National Hospital (KNH) and nurse midwives of the study team provided 24-h care to facilitate this process. At delivery, a standard form was used to collect delivery data and a cord blood sample.

2.2.4. Follow-Up

The mother-baby pairs were followed up with at 6, 10, and 14 weeks, and then monthly until the child was 1 year old, and thereafter every 3 months until 24 months of age or death of the infant. Blood samples were collected at pre-determined time points during the follow-up for HIV testing.

At each visit, interviews were conducted, and information was gathered using a standard tool on any symptoms of illness since the previous encounter and during the current visit, and of any illness or hospital visits/admissions in the period since the last clinic visit. This was done regardless of whether it was a scheduled or unscheduled visit due to illness. If the participants had sought medical care elsewhere, the take-home medical records or discharge summaries were examined, and information was abstracted to ensure completeness of documentation for the morbidities. The mother-baby pairs underwent a physical examination, their clinical characteristics were documented using a standard tool and the relevant study samples were collected by the team of study doctors, which included an obstetrics registrar and three consultants, two of whom are pediatricians, and one who is a medicine-pediatrics specialist.

To capture all morbidity events, the participants had unrestricted access to care in the study clinic that operated for 4 days a week and they received careful instructions on how to navigate the hospital for other services outside clinic hours. The study participants received a standard transport reimbursement

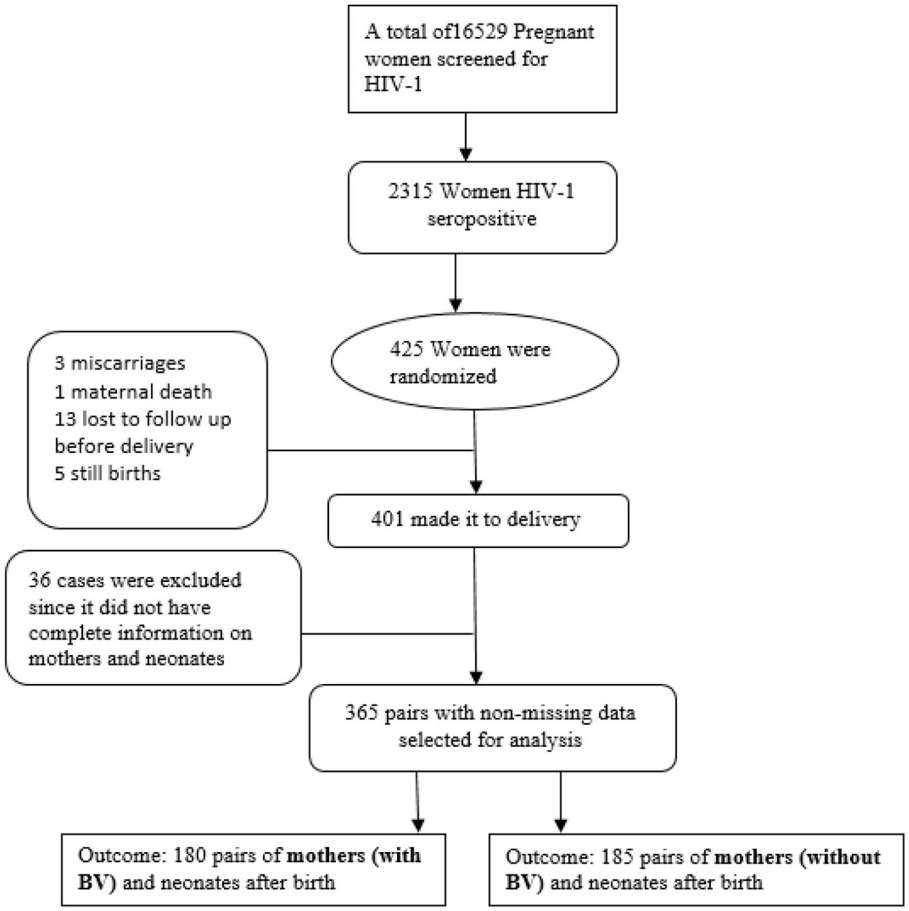

FIGURE 1 | Trial flow diagram describing the recruitment and exclusion of cases.

for every visit. The women were encouraged to attend the clinic from where they were enrolled for the child's follow-up, and to make sure the child received all the scheduled immunizations.

The primary intention-to-treat analysis was published in 2000, and the key findings were as follows: cumulative risk of infant infection at 24 months was 36.7% in the breastfeeding arm and 20.5% in the formula arm, and the estimated absolute risk of breastmilk transmission was 16.2 with 75% of the risk difference between the two arms of the study being achieved by 6 months (21). HIV-free survival in the breastfeeding and formula arms were 58.0 and 70.0%, respectively ($p = 0.02$). The 2-year cumulative mortality of the children was 24.4 and 20.0% in the breastfeeding and formula arms, respectively ($p = 0.30$) (21, 24). HIV-infected children have a 9-fold increased risk of death, and, of note, the incidence of diarrhea and pneumonia were identical in both arms of the study.

2.3. Clinical Characteristics During Follow-Up/Data Used for This Secondary Analysis

For purposes of this study, the following data were retrieved from the main dataset.

2.3.1. Mothers

We abstracted data on demographic characteristics (age, education, marital status), past reproductive health (number of pregnancies, live births), baseline viral load, whether they had BV or not, and delivery-related complications (excessive bleeding, urinary tract infection, hypertension), as well as the history and duration of hospitalization.

2.3.2. Newborns

We abstracted information on their status at delivery including, anthropometric measurements [length (cm), head circumference (cm), weight (g)], Apgar score at 1 and 5 min after birth, and estimated gestational age using the Dubowitz scores. Newborns with an estimated gestation of >37 completed weeks were classified as premature. Any breathing issues were classified as respiratory distress. Information was abstracted on whether the newborn had jaundice, conjunctivitis, lymphadenopathy, a skin rash, respiratory distress, or any other abnormalities after birth.

2.3.3. Infant Status During Follow-Up

Data was abstracted on presence of common childhood morbidities, including but not limited to pneumonia, ear

infection, blood in stool, lymphadenopathy, encephalopathy, sepsis, conjunctivitis, dehydration, wheezing, hematologic conditions, cold, otitis, fever, cough, diarrhea, thrush, vomiting, difficulty in feeding, heat rash, fungal rush, eczema/dermatitis, scabies, and mouth ulcer.

3. LABORATORY METHODS FOR DETECTION OF BV

Women were categorized as having BV or not using the Nugent criterion (25) as described in the original study.

4. ETHICAL APPROVAL

The study protocol was approved by the ethics review boards of the University of Washington and the University of Nairobi.

5. STATISTICAL ANALYSIS

Women who had BV and their exposed infants were compared to those who did not have BV. Mean values were used for characteristics with normal distribution, medians for the skewed data, and proportions for the categorical data. Effects of BV were measured and reported as a relative risk (RR) or odds ratio (OR).

To estimate the risk of BV of mothers during birth on (1) whether they needed hospital admission after facility delivery and (2) had adverse maternal condition, we estimated the RR.

We assessed the viral load data for normality using the histogram and found that it violated the bell shape required for normal distribution; therefore, we calculated the median viral load of 39,482 copies per ml. We then assessed the women with a viral load count more than the median, and less than the median, using chi-square statistics to check for statistical significance. We performed a log transformation of the viral load data to determine whether there was an association of the BV with HIV and used t-statistics to calculate the *P*-value to check for significance.

We compared BV-exposed and unexposed infants. For continuous data, we performed normality checks through standardized normal probability plots. Curves showing the bell shape of the normal distribution were considered normal data, and a *t*-test was performed, whereas curves that deviated from the assumption were considered non-normal data, and the non-parametric and Mann-Whitney *U* tests were performed to determine any associations. All tests were two-tailed. For the normal data, we reported data as the mean and standard deviation, whereas for the non-normal data, we reported data as the median and inter-quantile range.

We subsequently analyzed all the morbidity incidences ever reported on the infants between the two groups using Pearson's chi-squared test and computed the *p*-values using the Fisher's exact test. For the infant data obtained at 6 and 12 months after birth, we used the Generalized Estimating Equation (GEE) because the measures were repeated, and we had to control for the correlation. We employed the independence correlation structure, according to Hardin and Hilbe (26), with an assumption that the correlation among time points of infection is independent.

The inclusion of all the morbidities in the model was supported by the fact that some morbidities that were not significant or associated with BV were significant in the multiple logistic regression. Additionally, we adopted the suppressor effect concept proposed by Sun et al. (27) and argue that the methodological approach could show some patterns of the disease, which the conventional approaches fail to reveal.

Finally, to assess the effects of BV on survival between the two groups, we estimated the cumulative hazard using the Kaplan–Meier method. Our data met the two most important assumptions for this approach, namely: (1) censored subjects had the same risk as those remaining in the analysis; thus, censoring was non-informative; and (2) timing of exposure was known exactly; thus, infants exposed to BV were known before birth by checking their mothers' BV status. All cases of deaths from birth were included in the analysis.

All statistical analyses were performed using R version 3.6.3 (R Development Core Team, Vienna, Austria) (28). Analysis items with $P < 0.05$ were considered statistically significant.

6. RESULTS

6.1. Demographic Characteristics of the Women

The mean age was 23.5 years (range 17–39) for the BV-exposed infants and 24.1 years (15–38) for the unexposed infants. Among BV-exposed women 27.2% had not completed the 8 years of primary education, 19.6% had completed 8 years, and 53.3% had >8 years of education compared to 35.9, 17.2, and 46.9%, respectively among the unexposed women, however these differences were not significant ($p = 0.18$). The majority of the women were married, 81% of the BV-exposed group and 73.4% of the unexposed group, and these differences were not significant ($p = 0.18$) (**Table 1**).

6.2. Clinical Characteristics of Women Participants

The women had a mean of two pregnancies and a median of one live birth. Among the BV-exposed women 39.7, 47.3, and 13.0%, 0, 1–2, and > 3 previous live birth vs. 39.1, 44.8, and 16.1% among unexposed women. These differences were not significant ($p = 0.74$). Furthermore, 28.3% of the BV-exposed women reported having had an STD compared to 30.7% of the unexposed women, showing a non-significant difference ($p = 0.71$) (**Table 1**).

A total of 79 (53%) BV-exposed women and 76 (46%) unexposed women had a high viral load, higher than the calculated median (39482); the association of a higher viral load with BV was not statistically significant (RR = 1.15, CI = 0.92, 1.44, $p = 0.25$). The mean log viral load was 10.64 and 10.37 copies per ml for women afflicted with BV and those who were not, respectively, but results were not significant ($p = 0.19$) as shown in **Table 1**.

TABLE 1 | Comparison of the mothers demographic and selected maternal characteristics between the two groups.

Variable	Exposed	Unexposed	RR (95% CI)	*P*-value
MATERNAL DEMOGRAPHICS				
Age: Mean (Range)	23.5 (17–39)	24.1 (15–38)		0.21
Marital status: *N* (%)				
Single	28 (15.2%)	42 (21.9%)		0.18
Divorced/Separated/Widowed	7 (3.8%)	9 (4.7%)		
Married	149 (81.0%)	141 (73.4%)		
Education: *N* (%)				
<8 years	50 (27.2%)	69 (35.9%)		0.18
8 years complete	36 (19.6%)	33 (17.2%)		
>8 years	98 (53.3%)	90 (46.9%)		
Number of pregnancies: Mean (Range)	2 (1–8)	2 (1–6)		0.22
Number of live births: *N* (%)				
0	73 (39.7%)	75 (39.1%)		0.74
1 and 2	87 (47.3%)	86 (44.8%)		
>3	24 (13.0%)	31 (16.1%)		
Birth outcome: *N* (%)				
Live	168 (97.7%)	181 (98.4%)		0.31
Intra-partum deaths	2 (1.2%)	3 (1.6%)		
Still births	2 (1.2%)	0 (0%)		
Any STD: *N* (%)				
Yes	52 (28.3%)	59 (30.7%)		0.71
No	132 (71.7%)	133 (69.3%)		
MATERNAL CHARACTERISTICS				
Log viral load per milliliters: Mean (SD)	10.64 (1.79)	10.37 (1.88)		0.19
Viral load > 39, 482: *N* (%)				
Yes	79 (53.3%)	76 (46.3%)	1.15 (0.92–1.44)	0.25
No	69 (46.7%)	88 (53.7%)		
Admitted after birth?: *N* (%)				
Yes	32 (19.3%)	16 (9.7%)	1.99 (1.14–3.48)	0.02
No	134 (80.7%)	149 (90.3%)		
Adverse maternal conditions?: *N* (%)				
Yes	16 (9.7%)	7 (4%)	2.45 (1.04–5.81)	0.04
No	149 (90.3%)	170 (96%)		

6.3. Post-delivery Maternal Morbidity

Maternal conditions were reported by 23 women, including 16 (10%) of the 165 BV-exposed women and seven (4%) of the 177 unexposed women. The risk for an adverse maternal condition was significantly associated with BV RR = 2.45 [(95% CI 1.04, 5.81) $p = 0.04$] (**Table 1**).

6.4. Maternal Hospital Admission

Among 331 women who delivered in hospital, 48 (15%) required extended hospital admission, 19.3% were BV exposed, and 9.7% were unexposed to BV, RR = 1.99 [(95% CI 1.14–3.48), $P = 0.02$] as shown in **Table 1**. Forty-two women required admission for more than 1 day, including 21 (12.6%) of the 166 BV-exposed women and 15 (9.1%) of the 165 unexposed women; the difference was not significant ($p = 0.3$). BV-exposed women had a longer duration of admission compared to unexposed women, a higher maximum admission of 21 days against 16 for the unexposed. The mean hospital admission duration was 1.3 ± 3.2 days among women with BV and 0.7 ± 2.7 days among women with BV ($p = 0.91$).

6.5. Characteristics of the Neonates

Eighty-one (46.5%) of the 174 BV-exposed infants and 99 (55.5%) of the 179 unexposed infants, respectively, were male. The mean head circumference in both groups was 35.2 cm. BV-exposed babies were lighter than the unexposed infants, 3,096 vs. 3,196 g, an important trend on statistical testing ($p = 0.08$). In addition, five (3%) of the infants exposed to BV had a LBW (<2,500 g) compared to one (1%) in the unexposed infants ($p = 0.2$). BV-exposed and unexposed babies were of comparable length, and gestation. In addition, BV-exposed and unexposed neonates had comparable prevalence of neonatal morbidities that included jaundice (10.1 vs. 8.5%, $p = 0.76$), conjunctivitis (7.1 vs. 5.2%, $p = 0.61$ lymphadenopathy (6.5 vs. 9.1%, $p = 0.76$), respiratory

TABLE 2 | Neonatal characteristics and morbidities after birth.

Variable	Exposed Mean (SD)/*N* (%)	Unexposed Mean (SD)/*N* (%)	RR (95%CI)	*P*-value
NEONATAL CHARACTERISTICS				
Sex: *N* (%)				
Female	93 (53.5%)	79 (44.5%)		0.11
Male	81 (46.5%)	99 (55.5%)		
Maturity: Mean (SD)	39.5 (SD: 2.35)	40.0 (SD: 2.03)		0.26
Head circumference: Mean (SD)	35.2 (SD: 1.49)	35.3 (SD: 1.59)		0.88
Birth weight (in grams): Mean (SD)	3096 (SD: 547)	3196 (SD: 498)		0.08
Length: Mean (SD)	48.1 (SD: 4.3)	48.6 (SD: 2.45)		0.30
>37 completed gestation weeks: *N* (%)				
Yes	103 (92.8 %)	114 (95 %)	0.98 (0.92–1.04)	0.67
No	8 (7.2 %)	6 (5 %)		
Low birth weight: *N* (%)				
Yes	5 (3%)	1 (1%)	5.33 (0.63–45.11)	0.19
No	164 (97%)	179 (99%)		
Dead: *N* (%)				
Yes	9 (5%)	5 (3%)	1.85 (0.63–5.41)	0.39
No	171 (95%)	180 (97%)		
NEONATAL MORBIDITIES				
Jaundice: *N* (%)				
Yes	17 (10.1%)	15 (8.5%)	1.18 (0.61–2.29)	0.76
No	152(89.9%)	161(91.5%)		
Conjunctivitis: N (%)				
Yes	12 (7.1%)	9 (5.2%)	1.36 (0.59–3.16)	0.61
No	158 (92.9%)	165 (94.8%)		
Lymphadenophathy: *N* (%)				
Yes	11 (6.5%)	15 (9.1%)	0.72 (0.34–1.52)	0.51
No	158 (93.5%)	151 (90.9%)		
Respiratory distress: *N* (%)				
Yes	2 (1.2%)	2 (1.2%)	1.01 (0.14–7.10)	0.99
No	168 (98.8%)	170 (98.8%)		
Skin rash: *N* (%)				
Yes	20 (11.8%)	22 (12.6%)	0.94 (0.53–1.65)	0.94
No	150 (88.2%)	153 (87.4%)		
Other abnormalities after birth: *N* (%)				
Yes	5 (2.9%)	4 (2.3%)	1.28 (0.34–4.68)	0.97
No	165 (97.1%)	170 (97.7%)		

distress (1.2% each, $p = 0.9$), and skin rash (11.8 vs. 12.6%, $p = 0.94$) (**Table 2**).

6.6. Characteristics of the Infants

We first evaluated the "ever had" morbidity incidence, in which any infant who recorded any incidence was analyzed. In the test of association between BV and the various morbidities, only hepatomegaly and having a cold showed statistical significance. No other morbidities assessed showed any association with BV at $P < 0.05$. Using GEE to control for repeated events, the odds of morbidity at 6 and 12 months of infants exposed and unexposed to BV are shown in **Table 3**.

At 6 months of age, infants exposed to BV had significantly higher odds of reporting gastrointestinal symptoms of illness, including symptoms of passing bloody stool [OR 3.08 (1.11, 10.00), $p = 0.04$] and vomiting OR 1.64 (1.06, 2.56), $p = 0.03$], signs of dehydration [OR 2.94 (1.44, 6.37), $p = 0.01$], and mouth ulcers [OR 12.8 (2.27, 241.21), $p = 0.02$]. Babies exposed to BV were less likely to have lymphadenopathy [OR 0.74 (0.55, 0.99), $p = 0.04$] and fever [OR 0.75 (0.57, 0.99), $p = 0.04$] (**Table 3**).

At 12 months, there was a substantial decrease in the number of gastro-intestinal morbidities. Although, the exposed group had higher odds for the same, the trends for dehydration [OR 1.81 (1.05, 3.19), $p = 0.03$] and vomiting [OR 1.39 (1.01, 1.92), $p = 0.04$)] decreased. Babies exposed to BV were

TABLE 3 | Predictors of bacterial vaginosis at 6 and 12 months with corresponding 95% Confidence Intervals (CI) and *p*-values.

		6 months		12 months	
		OR (95% CI)	***P*-value**	**OR (95% CI)**	***P*-value**
Respiratory infections	Pneumonia	1.14 (0.75–1.72)	0.54	1.20 (0.88–1.65)	0.25
	Ear infection	0.55 (0.18–1.54)	0.27	0.63 (0.33–1.17)	0.14
	Wheezing	0.67 (0.34–1.27)	0.22	0.87 (0.59–1.27)	0.47
	Cold	1.09 (0.88–1.35)	0.44	0.99 (0.84–1.16)	0.88
	Otitis	1.10 (0.60–2.01)	0.76	1.10 (0.79–1.55)	0.57
	Cough	0.97 (0.77–1.23)	0.81	0.93 (0.78–1.10)	0.39
Gastrointestinal infections	Stool with blood	3.08 (1.11–10.00)	0.04	1.19 (0.67–2.12)	0.56
	Dehydration	2.94 (1.44–6.37)	0.01	1.81 (1.05–3.19)	0.03
	Diarrhea	0.72 (0.43–1.22)	0.23	0.64 (0.45–0.89)	0.01
	Vomiting	1.64 (1.06–2.56)	0.03	1.39 (1.01–1.92)	0.04
	Mouth ulcers	12.8 (2.27–241.21)	0.02	2.34 (1.00–6.03)	0.06
Other infections	Lymphadenopathy	0.74 (0.55–0.99)	0.04	0.65 (0.52–0.82)	0.01
	Encephalopathy	0.55 (0.07–3.57)	0.53	0.51 (0.07–2.74)	0.45
	Sepsis	1.27 (0.55–2.96)	0.57	1.18 (0.53–2.65)	0.68
	Conjunctivitis	1.32 (0.84–2.08)	0.24	1.41 (0.95–2.10)	0.09
	Difficulty feeding	0.74 (0.49–1.12)	0.16	0.78 (0.63–0.97)	0.02
	Heat rash	0.79 (0.55–1.13)	0.2	0.75 (0.56–1.01)	0.06
	Fungal rash	1.04 (0.62–1.77)	0.87	1.15 (0.75–1.78)	0.51
	Eczema/dermatitis	0.95 (0.76–1.19)	0.67	0.87 (0.73–1.04)	0.13
	Scabies	0.78 (0.37–1.62)	0.5	1.04 (0.69–1.55)	0.86
	Hepatomegaly	0.47 (0.19–1.05)	0.08	0.56 (0.32–0.94)	0.03
	Fever	0.75 (0.57–0.99)	0.04	0.89 (0.73–1.07)	0.22
	Thrush	0.94 (0.64–1.37)	0.74	0.92 (0.67–1.28)	0.63

less likely to have lymphadenopathy [OR 0.65 (0.52, 0.82), $p = 0.01$], difficulty in feeding [OR 0.78 (0.63, 0.97)], and hepatomegaly [OR 0.56 (0.32, 0.94), $p = 0.03$]. The other characteristics assessed were not significant at the 0.05 level (**Table 3**).

BV-exposed and unexposed infants had similar incidence of conditions affecting the respiratory and other systems at 6 and 12 months.

7. MORTALITY IN THE FIRST 12 MONTHS OF LIFE

In the BV-exposed pregnancies, there were two still births and two intrapartum deaths, in contrast to zero still births and three intrapartum deaths. Over the first year, there were 14 deaths in the first year, (9 [5%]) of 180 in the exposed group, and (5 [2.8%]) in the 185 unexposed group, showing a risk difference of 2.2%. The relative risk of death in the exposed group was RR = 1.85 [(95% CI 0.63–5.41), $p = 0.39$]. We compared the survival between infants whose mothers were exposed to BV and those whose mothers were not, using the KM (**Figure 2**). The graph showed a trend of higher mortalities in the BV-exposed group. The difference was not statistically significant ($p = 0.65$).

8. DISCUSSION

In this study, we examined the association of maternal BV with a mothers' condition and infants' morbidities. Our analysis showed an increase in the frequency of maternal hospital admissions among women with BV compared to non-exposed subjects. In our literature review, we did not find any publication that directly reports on maternal perinatal adverse outcome associated with BV, and reports have universally focused on fetal/neonatal outcome. The closest article was a systematic review on the use of probiotics to improve maternal microbiota, which demonstrated a reduction in gestational diabetes and better blood sugar control. Prolonged hospital admission has a cost implication and increases the risk of nosocomial infections (29).

Exposure to BV did not show any association with any neonatal morbidities, as previously reported (21). However, the lower mean birth weight in the exposed group in this study was in line with the findings reported by others (30–32). Women who were diagnosed with BV were provided treatment in the third trimester of pregnancy, and this may have attenuated the differences between BV-exposed and unexposed women.

The most novel observation from this study was the link between BV and morbidities among infants at 6 and 12 months after birth. The children exposed to BV had significantly more symptoms of gastrointestinal diseases. Additionally, there was

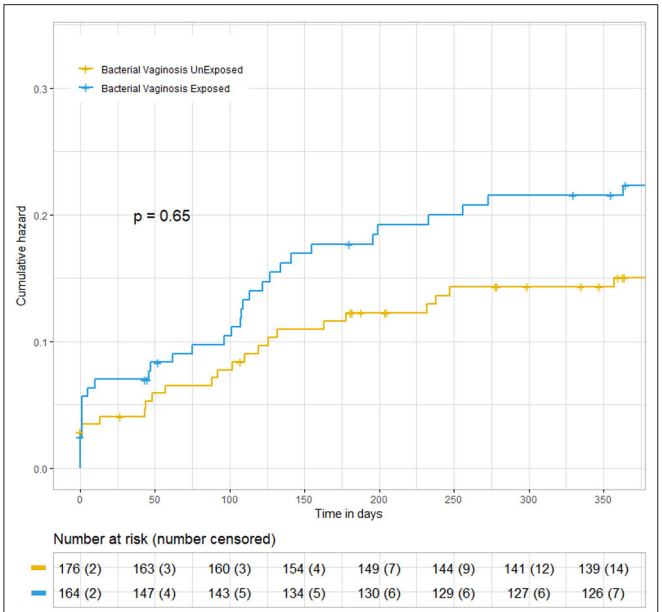

FIGURE 2 | Kaplan–Meier analysis of infant mortality over 12 months between infants with and without maternal exposure to bacterial vaginosis.

increased mortality among infants exposed to BV, although, the latter did not achieve statistical significance. These observations support our hypothesis that babies exposed to BV have Lactobacillus-deficient biota characterizing BV, making them more vulnerable to gastrointestinal infections (33). It is prudent to note that these effects were more commonly observed in the first 6 months of life and decreased as the baby grew older. This is supported by our previous analysis of this data that reported a decrease in morbidities with time (34).

Fouhy et al. in the 2012 comprehensive review of the composition of early intestinal microbiota observe that the gut is sterile at birth and is progressively colonized by bacteria achieving an adult like microbiome by the age of 2 (35). The initial colonization of the gut influences the subsequent development of the immune system, including the gut associated lymphoid tissues and that gut biota play a role in the regulation of the immune system. It is further postulated that an altered gut biota composition may predispose the infant to infections and allergic reactions. In this study we show that BV-exposed babies have increased gastrointestinal infections. Consistent with the concept of a maturing gut microbiota, the incidence of the infections diminished as the infants advanced in age.

Studies contrasting babies born vaginally with those born by caesarian section show that the gut is first colonized with the mother's vaginal and fecal microbes, which quickly helps to establish the dominant number of lactobacillus within a few hours of delivery. In contrast, Cesarean babies are first colonized with maternal skin microbes with a predominance of staphylococci. It is reasonable to assume that BV-exposed infants were colonized with bacteria typical of vaginal dysbiosis (35).

A number of studies have looked at the long-term impact of being born vaginally vs. Cesarean, therefore acquiring different gut biota, and found worse outcomes in allergy and metabolic conditions, such as asthma and obesity among those born by Cesarean (35). We have not found any studies reporting on the gut biota of babies born to women with vaginal dysbiosis. BV is best known for its role in increasing risk of prematurity. Based on the findings of this study, there is a need to further evaluate its contribution to infant infections and mortality.

Unexposed subjects had higher odds of manifesting lymphadenopathy, hepatomegaly, difficulty feeding, and fever, and may reflect reverse causality. The BV-exposed children may have had greater access to antimicrobial therapy, which would have treated a variety of illness beyond what they were reported to have, while the unexposed children went on to have a full expression of their illness.

There has not been any published literature linking child mortality directly to BV. However, there is an indirect link through the related adverse pregnancy outcome, including preterm birth, prematurity, and pregnancy complications, which are factors directly related to neonatal and infant mortality (36). Our Kaplan–Meier analysis, through the cumulative hazard plot, showed a non-significant difference ($p = 0.65$); however, the graph showed a trend toward a higher mortality rate in the BV-exposed group. Therefore, this means that the risk of mortality still exists among infants whose mothers are exposed to BV. Our study was not powered to fully evaluate this. Future studies regarding BV and mortality are needed to confirm our results and to elucidate the underlying associations.

We believe the results of our study are credible. The diagnosis of BV was made using the Nugent method, the gold standard, and therefore, there is clarity on the infants' exposure. The technologist who examined the slides was part of the team that developed the Nugent method for diagnosing BV. We used the Nugent method to diagnose BV, which has the advantage of having a standardized scoring system, and therefore, reduces variability in reporting. The alternative method would have been to culture the bacteria, but at the time, there was limited microbiology culture capacity, and diagnosis of BV was not the primary endpoint of the study. Since then, the development of the field of genomics has expanded on opportunities to describe the microbiota, and in the process, has expanded the spectrum of bacteria identified to be part of the human microbiome (10, 13). Studies that have evaluated these newer techniques along with the newer genomic studies have found congruent study findings (35).

The infants were seen frequently for both scheduled and unscheduled visits as needed, and a standard tool was used to collect the data, and therefore, we are confident regarding the documentation about the presence or absence of a specific morbidity. The clinics were run by very qualified staff; thus, the data collected was of high quality, there were few cases with missing data, and therefore, the results are generalizable. For example, of the 401 mother and infant pairs in the original study, the prevalence of BV as reported by Nduati et al. (21) was 47%. Subsequently, on the final pairs included in our analysis, the prevalence of BV was calculated as 48% (159/328). This is consistent with the findings of other studies that reported the prevalence of BV in Kenya to be between 30 and 50% (37). In

terms of methodology, the use of GEE is based on its unique property of handling repeated measures.

In this study, the syndromic treatment of women with abnormal vaginal discharge, which covers for BV, and prompts the treatment of children's illness, means that effects of BV are attenuated toward null. As observed earlier, participants received transport reimbursement and the research clinic provided a safe space when HIV was still new and very stigmatized. Both BV-exposed and unexposed babies had a median number of eight visits during the first year of life. With this, we were not able to establish the correct frequency of hospital visits due to genuine morbidities.

9. CONCLUSIONS AND FUTURE RESEARCH

This paper reported many findings that are consistent with those reported in the literature and has added further knowledge in the area of BV. While previous studies have focused only on the effects of BV on infants, our study explored the effects of BV on both the mother and baby in the context of HIV. This has an implication for infants who are more vulnerable to several infections due to a compromised immune status. Our study has shown independence in terms of association of BV with HIV and recommends for BV to be researched alone. Future research is recommended to gain deeper insights into the predictors of BV.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://osf.io/yxbhg/files/.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by University of Nairobi and Washington Institution Review Board. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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REFERENCES

1. Haggerty CL, Hillier SL, Bass DC, Ness RB, Evaluation PID, Investigators CHPS. Bacterial vaginosis and anaerobic bacteria are associated with endometritis. *Clin Infect Dis*. (2004) 39:990–5. doi: 10.1086/423963
2. Parnell LA, Briggs CM, Mysorekar IU. Maternal microbiomes in preterm birth: recent progress and analytical pipelines. *Semin Perinatol*. (2017) 41:392–400. doi: 10.1053/j.semperi.2017.07.010
3. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. *Lancet*. (2008) 371:75–84. doi: 10.1016/S0140-6736(08)60074-4
4. Ezechi OC, David AN, Gab-Okafor CV, Ohwodo H, Oladele DA, Kalejaiye OO, et al. Incidence of and socio-biologic risk factors for spontaneous preterm birth in HIV positive Nigerian women. *BMC Pregnancy Childbirth*. (2012) 12:93. doi: 10.1186/1471-2393-12-93
5. Hillier SL, Nugent RP, Eschenbach DA, Krohn MA, Gibbs RS, Martin DH, et al. Association between bacterial vaginosis and preterm delivery of a low-birth-weight infant. *N Engl J Med*. (2018) 333:1737–42. doi: 10.1056/NEJM199512283332604
6. Slyker JA, Patterson J, Ambler G, Richardson BA, Maleche-Obimbo E, Bosire R, et al. Correlates and outcomes of preterm birth, low birth weight, and small for gestational age in HIV-exposed uninfected infants. *BMC Pregnancy Childbirth*. (2014) 14:7. doi: 10.1186/1471-2393-14-7
7. WHO. *Born Too Soon: The Global Action Report on Preterm Birth*. Geneva: WHO (2012).
8. Lawn JE, Gravett MG, Nunes TM, Rubens CE, Stanton C, The GAPPS Review Group. Global report on preterm birth and stillbirth (1 of 7): definitions, description of the burden and opportunities to improve data. *BMC Pregnancy Childbirth*. (2010) 10:S1. doi: 10.1186/1471-2393-10-S1-S1
9. Dingens AS, Fairfortune TS, Reed S, Mitchell C. Bacterial vaginosis and adverse outcomes among full-term infants: a cohort study. *BMC Pregnancy Childbirth*. (2016) 16:278. doi: 10.1186/s12884-016-1073-y
10. Redelinghuys MJ, Geldenhuys J, Jung H, Kock MM. Bacterial vaginosis: current diagnostic avenues and future opportunities. *Front Cell Infect Microbiol*. (2020) 10:354. doi: 10.3389/fcimb.2020.00354
11. Ñahui Palomino RA, Zicari S, Vanpouille C, Vitali B, Margolis L. Vaginal lactobacillus inhibits HIV-1 replication in human tissues *ex vivo*. *Front Microbiol*. (2017) 8:906. doi: 10.3389/fmicb.2017.00906
12. Yuh T, Micheni M, Selke S, Oluoch L, Kiptinness C, Magaret A, et al. Sexually transmitted infections among Kenyan adolescent girls and young women with limited sexual experience. *Front Public Health*. (2020) 8:303. doi: 10.3389/fpubh.2020.00303
13. Gupta P, Singh MP, Goyal K. Diversity of vaginal microbiome in pregnancy: deciphering the obscurity. *Front Public Health*. (2020) 8:326. doi: 10.3389/fpubh.2020.00326
14. Bukusi EA, Cohen CR, Meier AS, Waiyaki PG, Nguti R, Njeri JN, et al. Bacterial vaginosis: risk factors among Kenyan women and their male partners. *Sex Transm Dis*. (2006) 33:361–7. doi: 10.1097/01.olq.0000200551.07573.df
15. Torrone EA, Morrison CS, Chen PL, Kwok C, Francis SC, Hayes RJ, et al. Prevalence of sexually transmitted infections and bacterial vaginosis among women in sub-Saharan Africa: an individual participant data meta-analysis of 18 HIV prevention studies. *PLoS Med*. (2018) 15:e1002511. doi: 10.1371/journal.pmed.1002511
16. Atashili J, Poole C, Ndumbe PM, Adimora AA, Smith JS. Bacterial vaginosis and HIV acquisition: a meta-analysis of published studies. *AIDS*. (2008) 22:1493–501. doi: 10.1097/QAD.0b013e3283021a37
17. Mirmonsef P, Krass L, Landay A, Spear GT. The role of bacterial vaginosis and trichomonas in HIV transmission across the female genital tract. *Curr HIV Res*. (2012) 10:202–10. doi: 10.2174/157016212800618165
18. John GC, Nduati RW, Mbori-Ngacha DA, Richardson BA, Panteleeff D, Mwatha A, et al. Correlates of mother-to-child human immunodeficiency virus type 1 (HIV-1) transmission: association with maternal plasma HIV-1 RNA load, genital HIV-1 DNA shedding, and breast infections. *J Infect Dis*. (2001) 183:206–12. doi: 10.1086/317918

19. Farquhar C, Mbori-Ngacha D, Overbaugh J, Wamalwa D, Harris J, Bosire R, et al. Illness during pregnancy and bacterial vaginosis are associated with *in-utero* HIV-1 transmission. *AIDS*. (2010) 24:153–5. doi: 10.1097/QAD.0b013e32832326d8
20. Warren D, Klein RS, Sobel J, Kieke J B, Brown W, Schuman P, et al. A multicenter study of bacterial vaginosis in women with or at risk for human immunodeficiency virus infection. *Infect Dis Obstetr Gynecol*. (2001) 9:133–41. doi: 10.1155/S1064744901000242
21. Nduati R, John G, Mbori-Ngacha D, Richardson B, Overbaugh J, Mwatha A, et al. Effect of breastfeeding and formula feeding on transmission of HIV-1: a randomized clinical trial. *JAMA*. (2000) 283:1167–74. doi: 10.1001/jama.283.9.1167
22. Fouda GG, Martinez DR, Swamy GK, Permar SR. The impact of IgG transplacental transfer on early life immunity. *Immunohorizons*. (2018) 2:14–25. doi: 10.4049/immunohorizons.1700057
23. Nguyen DTN, Hughes S, Egger S, LaMontagne DS, Simms K, Castle PE, et al. Risk of childhood mortality associated with death of a mother in low-and-middle-income countries: a systematic review and meta-analysis. *BMC Public Health*. (2019) 19:1281. doi: 10.1186/s12889-019-7316-x
24. Mbori-Ngacha D, Nduati R, John G, Reilly M, Richardson B, Mwatha A, et al. Morbidity and mortality in breastfed and formula-fed infants of HIV-1-infected women: a randomized clinical trial. *JAMA*. (2001) 286:2413–20. doi: 10.1001/jama.286.19.2413
25. Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. *J Clin Microbiol*. (1991) 29:297–301. doi: 10.1128/JCM.29.2.297-301.1991
26. Hardin JW. *Generalized Estimating Equations*. Hardin, MT; Boca Raton, FL: CRC Press (2013). doi: 10.1002/9781118445112.stat06428
27. Sun GW, Shook TL, Kay GL. Inappropriate use of bivariable analysis to screen risk factors for use in multivariable analysis. *J Clin Epidemiol*. (1996) 49:907–16. doi: 10.1016/0895-4356(96)00025-X
28. R Core Team. *R: A Language and Environment for Statistical Computing*. Vienna (2017). Available online at: https://www.R-project.org/
29. Qin S, Liu Y, Wang S, Ma J, Yang H. Distribution characteristics of intestinal microbiota during pregnancy and postpartum in healthy women. *J Matern Fetal Neonat Med*. (2021):1–8. doi: 10.1080/14767058.2020.1812571
30. Isik G, Demirezen S, Dönmez HG, Beksac MS. Bacterial vaginosis in association with spontaneous abortion and recurrent pregnancy losses. *J. Cytol*. (2016) 33:135–40. doi: 10.4103/0970-9371.188050
31. Thorsen P, Vogel I, Olsen J, Jeune B, Westergaard JG, Jacobsson B, et al. Bacterial vaginosis in early pregnancy is associated with low birth weight and small for gestational age, but not with spontaneous preterm birth: a population-based study on Danish women. *J Matern Fetal Neonat Med*. (2006) 19:1–7. doi: 10.1080/14767050500361604
32. Freitas AC, Chaban B, Bocking A, Rocco M, Yang S, Hill JE, et al. The vaginal microbiome of pregnant women is less rich and diverse, with lower prevalence of Mollicutes, compared to non-pregnant women. *Sci Rep*. (2017) 7:9212. doi: 10.1038/s41598-017-07790-9
33. Anigilaje EA. Management of diarrhoeal dehydration in childhood: a review for clinicians in developing countries. *Front Pediatr*. (2018) 6:28. doi: 10.3389/fped.2018.00028
34. Mwenda N, Nduati R, Kosgei M, Kerich G. Skewed logit model for analyzing correlated infant morbidity data. *PLoS ONE*. (2021) 16:e0246269. doi: 10.1371/journal.pone.0246269
35. Fouhy F, Ross RP, Fitzgerald GF, Stanton C, Cotter PD. Composition of the early intestinal microbiota: knowledge, knowledge gaps and the use of high-throughput sequencing to address these gaps. *Gut Microbes*. (2012) 3:203–20. doi: 10.4161/gmic.20169
36. Kenya National Bureau of Statistics NAIDSCCKMRI Ministry of Health/Kenya, NCfP, Development/Kenya. *Kenya Demographic and Health Survey 2014*. Rockville, MD (2015). Available online at: http://dhsprogram.com/pubs/pdf/FR308/FR308.pdf
37. Cohen CR, Lingappa JR, Baeten JM, Ngayo MO, Spiegel CA, Hong T, et al. Bacterial vaginosis associated with increased risk of female-to-male HIV-1 transmission: a prospective cohort analysis among African couples. *PLoS Med*. (2012) 9:e1001251. doi: 10.1371/journal.pmed.1001251

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Biological and Psychosocial Factors, Risk Behaviors, and Perinatal Asphyxia in a University Hospital: Matched Case–Control Study, Cali, Colombia (2012–2014)

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Introduction: Perinatal asphyxia is one of the main causes of morbidity and mortality in newborns. It generates high costs, both social and economic, and presents modifiable risk factors.

Objective: To determine the biological and psychosocial factors and risk behaviors associated with the development of perinatal asphyxia (Sarnat II-III) in newborns from low socioeconomic status in a tier III university hospital in the city of Cali, Colombia.

Materials and Methods: With a case and control design, 216 patients were studied (54 cases/162 controls) (1 case/3 matched controls). The cases were defined as newborns with modified or severe perinatal asphyxia (Sarnat II-III) between 2012 and 2014, with gestational age ≥ 36 weeks, with neurological signs not attributable to other causes, multiorgan compromise, advanced reanimation, and presence of a sentinel event. For the analysis, conditional logistic regression models were developed to evaluate association (OR), considering that the cases and controls had been paired by the birth and gestational age variables.

Results: The final model showed that, from the group of biological variables, meconium amniotic fluid was identified as a risk factor (OR 15.28, 95%CI 2.78–83.94). Induction of labor lowered the risk of perinatal asphyxia by 97% (OR 0.03, 95%CI 0.01–0.21), and monitoring of fetal heart rate was associated with lower odds by 99% (OR 0.01, 95%CI 0.00–0.31) of developing perinatal asphyxia in the newborn. Regarding social variables, the lack of social support was identified as a risk factor for the development of perinatal asphyxia (OR 6.44, 95%CI 1.16–35.66); in contrast, secondary education lowered the odds of developing perinatal asphyxia by 85% when compared with pregnant women who only had primary school education (OR 0.15, 95%CI 0.03–0.77).

Conclusion: Assessment of biological and psychosocial factors and social support is important in pregnant women to determine the risk of developing perinatal asphyxia in a low-income population.

Keywords: perinatal asphyxia, meconium bronchoaspiration, hypoxic-ischemic encephalopathy, logistic regression model, associated factors, cases and controls matched

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INTRODUCTION

Perinatal asphyxia is one of the main causes of perinatal and neonatal morbidity and mortality, mainly in low-income countries (1, 2). It is estimated globally that in full-term infants, occurrence of perinatal asphyxia is between 2 and 4 per 1,000 newborns; this estimate is higher in pre-mature children (3). Other studies report an incidence of 5–10 per 1,000 live births, with likely underreporting of this entity (4). In Colombia, this incidence is unknown. Perinatal asphyxia can be defined as a syndrome with a wide variety of clinical features in which newborns show specific neurological abnormalities during the first 24 h after birth. This is followed by acute events, characterized by cardiorespiratory depression leading to variable degrees of hypoxemia and hypercapnia and generating metabolic acidosis (5, 6). These events can occur around the time of birth due to fetal lack of oxygen and/or inadequate tissue perfusion (6). Asphyxia and the resulting hypoxic-ischemic encephalopathy are considered frequent causes of chronic disability, such as cerebral palsy, intellectual disability, learning disorders, and epilepsy. It is estimated that ∼15–20% of newborns that develop perinatal asphyxia have brain damage and die within the first 28 days of life. Of those who survive, 25% may have permanent neuropsychological sequelae (7, 8). All of this generates deleterious psychosocial effects in society and in the families of affected patients, in addition to high costs for the health system (9, 10).

Perinatal mortality in Latin America varies considerably from one region to another (South America and Mexico with 21.4 per 1,000 live births, Central America with 35.4 per 1,000 live births, and the Caribbean with 52.8 per 1,000 live births). In Colombia, a ratio of 24 deaths per 1,000 live births was identified, and for the department of Valle del Cauca, a ratio of 12.4 per 1,000 live births was identified (11). In the city of Cali, the third most populous city in Colombia, 57% of the total perinatal mortality rate corresponds to the neonatal component, the infant mortality rate (IMR) in 2008 was 13.4 deaths per 1,000 live births, and the neonatal component was of 64% (12). The aforementioned data reflect the magnitude of the problem in the studied population.

MATERIALS AND METHODS

Ethical Considerations

This study was undertaken with approval by the Ethics Committee at the University Hospital del Valle and Universidad del Valle.

Pregnant women who agreed to participate in the study granted authorization through an informed consent, according to Helsinki international ethical regulations.

Design

This was a retrospective analytical observational study, where cases and controls were matched by the gestational age and the type of delivery (vaginal delivery or cesarean section).

The main outcome was perinatal asphyxia, and independent variables were the maternal factors recorded from the clinical files of the infants and through interviews with the mothers of the patients.

Study Population

The study was conducted in a tier III hospital in the city of Cali, Colombia, a public institution with facilities for newborn care. In 2012, 5,300 births were attended, with 90% of these subsidized by the healthcare system. The newborns diagnosed with moderate (Sarnat II) and severe (Sarnat III) (13) perinatal asphyxia admitted to the institution, along with the newborns who according to the selection criteria were defined as controls, were considered as the study population. There were three controls for each case.

Definition of cases and controls: For participants, selection criteria and restrictions were applied in a matched case–control design. The criteria were defined in accordance with that established by the American College of Gynecology and Obstetrics and Its Pediatricians (6). The study population consisted of all newborns with gestational age $\geq$ 36 weeks, diagnosed with moderate (Sarnat II) and severe (Sarnat III) perinatal asphyxia, admitted to the hospital, or all neonates diagnosed with perinatal asphyxia during their stay in the unit, recognized as postnatal asphyxia (Sarnat II or III). Inclusion criteria considered patients with

1. Gestational age $\geq$ 36 weeks determined by amenorrhea, by early ultrasound, or by Ballard et al. (14)
2. Neonatal neurological signs (convulsion, coma, hypotonic) not attributable to another cause, confirmed by pediatrician evaluation
3. Arterial pH $\leq$ 7.0 or a base deficit of at least 12 mmol/L taken in the first hour of life
4. An Apgar score of 0–3 after 5 min
5. Multiorgan compromise (two or more organs) not attributable to another cause (central nervous system, renal, pulmonary, cardiovascular, gastrointestinal, metabolic, and hematological)
6. Need for advanced neonatal resuscitation
7. Presence of a sentinel event [uterine rupture, placental abruption, thromboembolism of amniotic fluid, and prolapse of the umbilical cord in a patient who also meets the essential criteria of metabolic acidosis (pH of cord <7 base deficit <-12 meq) and early neurological compromise]

Inclusion criteria for controls are as follows:

1. Not having developed perinatal asphyxia
2. A difference in age from the case of <1 week
3. Being born by the same means of delivery as the cases having gestational age comparable to cases of up to 1 week

The exclusion criteria were defined as follows:

1. Mild perinatal asphyxia in infants (Sarnat I), who developed a clinical spectrum at birth remarkably similar to normal children with sequelae <1%, according to that reported in the literature (15).
2. Patients with major congenital malformations or with congenital or explicable neurological alterations due to

a condition other than perinatal asphyxia (electrolyte disturbance, inborn error of metabolism). Such children were not included in the analysis because they were considered possible generators of confusion or information bias. To improve the efficiency of the study, it was necessary to increase the number of controls to three for each case. With the aforementioned and based on the study by Torres-Muñoz et al. (16) from 2010 to 2011, a relationship of three controls was designated for each case.

The sample size was determined considering the prevalence of perinatal asphyxia reported in previous studies (16) with an alpha error of 0.1 and a beta error of 0.20, exposure in the controls of 0.6%, and exposure of the cases of 12.7%. Considering that the cases were paired with controls for two variables (the type of delivery and the gestational age), it was decided to use the comparison formula of proportions for paired groups proposed by Connor in 1987 (17). Pregnant women who agreed to participate in the study signed an informed consent, designed according to required ethical standards. The cases were selected by intentional sampling, when meeting the established selection criteria, during the 2 years (2012–2014) of data collection.

The Medical Outcomes Study (MOS) Social Support Survey evaluated different measurements previously validated in Colombia (18). Questionnaires were administered during follow-up consultations of newborns in the participating institution, guaranteeing the privacy and tranquility of the mothers. Mother self-completed the questionnaires, with support from the care pediatrician prior to delivery who verified that all questions were answered. When a mother was illiterate or could not fill out the questionnaires on her own, she was helped by a pediatrician from the consultation or by the researchers.

The MOS-23 questionnaire is a self-administered form developed by Sherborne et al. (19) in 1991; each item is designed to evaluate

- Structural or quantitative support: 1 item
- Emotional/informational support, as an expression of affection and understanding: 8 items
- Social interaction, such as the availability of other people to meet: 4 items
- Affective support, with real love demonstrations: 3 items
- Instrumental support, material or tangible help received: 4 items

The items are answered in two different ways: four of them are of dichotomous response (yes or no), and the other two are answered according to a four-point Likert scale (0 to 3 points). The total score is obtained by adding the scores obtained in each item, and ranges from 0 to 10 points were considered. The score of this scale was adopted in this research.

Analysis Plan

To consolidate the information obtained, a form was designed in the Epi Info software version 3, where the data of biological and social variables, obtained through the clinical history of each patient, were entered. In this same form, the MOS questionnaire was also included to facilitate the collection of information and verification of the clinical history, taking advantage of the fact that the mother was present to complete the questionnaire. The data were recorded in an.xls file to be worked in Excel® and then exported to the STATA 13 software (Statacorp Inc. Texas) for statistical analyses.

Biological and social risk factors for the initial evaluation of the quantitative variables were carried out through univariate analysis in which the statistics of central tendency and dispersion were reported, according to the distribution of each variable. For the variables of maternal age, maternal weight, body mass index, and newborn weight, the Shapiro–Wilk-test was used to evaluate normality in the data distribution. Subsequently, a bivariate analysis was performed in which the strength of the association (exposure OR) was determined with respective 95% confidence intervals (95% CIs) between the dependent and independent variables (bivariate analysis), and contingency tables were generated. Regarding the categorical variables, an exploratory univariate analysis was conducted in which absolute frequencies were expressed, followed by a bivariate analysis using the chi-square test or the Fisher exact-test, as appropriate. The strength of the association (OR) and its 95% CI, between the dependent and independent variables, were determined. We determined the respective adjusted OR of each group of variables once the possible effects of interaction and confusion between the different factors and covariables considered were analyzed and discarded. For this purpose, a multiple conditioned logistic regression model was used bearing in mind that it was paired by birth and gestational age. To select the variables included in each of the models, a forward stepwise procedure was used. The conditioned logistic regression was chosen considering that, in this study, pairing was used to control for the type of the delivery and for the gestational age. The conditional logistic regression (CLR) is a specialized logistic regression, so coefficients, odds ratios, and adjustment statistics can be interpreted the same way as for ordinary logistic regression.

RESULTS

Initially, 311 newborns were admitted, of which 54 cases and 162 controls were selected (**Figure 1**). The general characteristics of the population in relation to the biological variables are described according to case or control (**Table 1**). Bivariate comparisons between the cases and the controls showed that not monitoring the fetal heart rate was found more often in the cases than in the controls ($p < 0.001$). Similar results were observed for not monitoring of labor, which was more frequent in the cases than in the controls ($p < 0.001$), and chorioamnionitis ($p < 0.01$). In contrast, oxytocin use was used more often in the controls than in the cases ($p < 0.001$). We observed that newborns considered as cases presented meconium amniotic fluid in 62.30 vs. 13.50% of the controls ($p < 0.001$). Of the babies diagnosed with perinatal asphyxia, 94.40% required resuscitation maneuvers, 90.70% required mechanical ventilation, 96.30% were diagnosed with multiorgan dysfunction, and 5.6% died.

Social variables are described in **Table 2**. Regarding educational attainment, only 48.20% of the cases but 76.5%

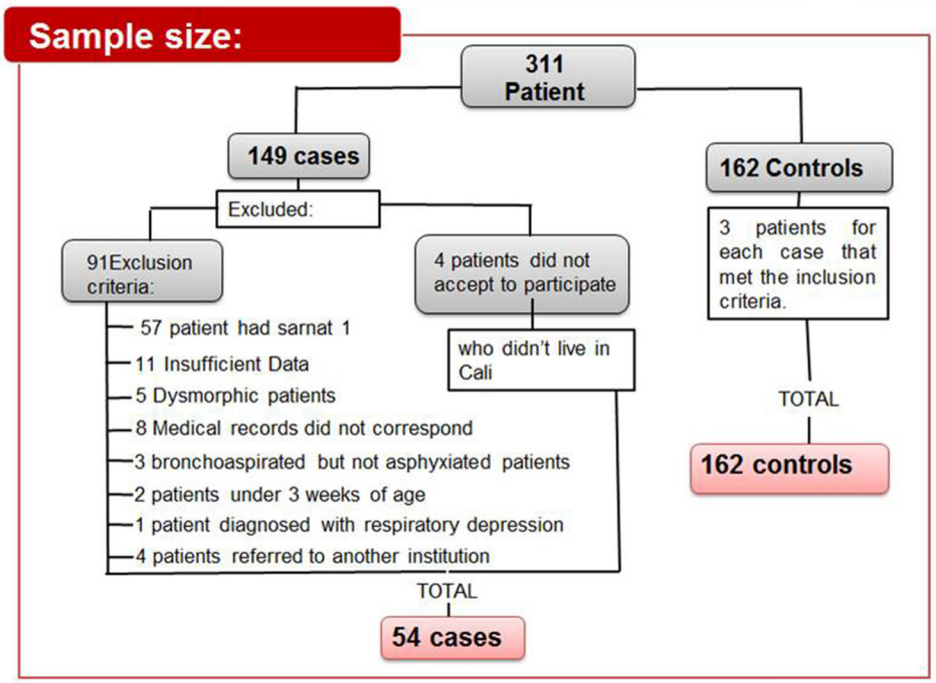

FIGURE 1 | Case selection scheme. Selection process of newborns from cases and controls.

of the controls had a high school education ($p < 0.001$). In the analysis of subscales with the MOS questionnaires, low support was noted in four categories: emotional subscale (33.30% cases vs. 16.70% controls) ($p < 0.001$); social subscale (22.60% cases vs. 0.60% controls) ($p < 0.001$); affective subscale (37.70% cases vs. 11.70% controls) ($p < 0.001$); and instrumental subscale (42.60% cases vs. 14.20% controls) ($p < 0.001$). In behavioral variables, only two of the cases and one of the controls smoked. Passive exposure to cigarette smoke was present in five cases, and consumption of psychoactive substances was present in three cases and one control. Models of conditioned logistic regression, including the type of delivery and the gestational age, allowed quantifying associations for each group of variables.

Regarding the odds of developing asphyxia (**Table 3**), a significant OR was found for several variables. Multiparity (defined as having more than one birth) was associated with the newborn not being suffocated with an odds of 63% (OR 0.37, 95%CI 0.19–0.72). Induction of labor was associated with lower odds of suffocation in 93% (OR 0.07, 95%CI 0.03–0.15). The odds of asphyxia in newborns from mothers with chorioamnionitis was 5.9 times greater (OR 5.9, 95%CI 1.05–33.44); when amniotic fluid had meconium, infants had an 11.7-fold higher risk of developing asphyxia (OR 11.71, 95%CI 5.46–25.14). Cases born from mothers with preeclampsia had a higher odds and had three times greater of developing perinatal asphyxia (OR 3.04, 95%CI 1.51–6.12); in this variable, confounder factors (the type of delivery and the gestational age) were identified, given that women with preeclampsia are more likely to deliver early *via* cesarean section.

Table 4 shows the conditioned OR of the possible explanatory social variables for perinatal asphyxia. Mothers that course secondary education have 68% less chances of having a baby with asfixia (OR 0.32, 95%CI 0.17–0.60), and those with ≥ prenatal visits had 75% lower odds of delivering infants with asphyxia (OR 0.25, 95%CI 0.12–0.51). Regarding the different subscales of the MOS questionnaire, a greater odds of developing asphyxia was observed in the deficient score in the subscales: emotional 2.5 times (OR 2.51, 95%CI 1.25–5.06), social 53.55 times (OR 53.55, 95%CI 6.66–430.05), affective 4.37 times (OR 4.37, 95%CI 2.08–9.20), and instrumental 4.17 times (OR 4.17, 95%CI 2.07–8.38).

In **Table 5**, after adjusting the model to explain the relationship between the dependent variable asphyxia and all the others, determining if the relationship is significant, the following results were generated: the odds of asphyxia in cases that presented with meconium amniotic fluid was 15.28 times higher compared with that of the controls (OR 15.28, 95%CI 2.78–83.94). Variables related to labor monitoring were also found to be significant in their association with perinatal asphyxia; the introduction of labor decreased the odds by 97% (OR 0.03, 95%CI 0.01–0.21), and the odds or likelihood of not developing asphyxia was lowered by monitoring the fetal heart rate by 99% (OR 0.01, 95%CI 0.00–0.31). Having schooling up to the secondary level decreased the odds of developing perinatal asphyxia by 85% compared to those who only studied until primary school (OR 0.15, 95%CI 0.03–0.77). When applying the MOS questionnaire, the instrumental subscale showed that without adequate support in the cases, the likelihood of developing asphyxia was 6.44 times higher in cases than in those with adequate support (OR 6.44, 95%CI 1.16–35.66). The variables chorioamnionitis, preeclampsia, and <4 prenatal visits were significant in the

TABLE 1 | Description of biological variables.

Characteristics	Cases (54) (%)	Controls (162) (%)	*P*-value**
Age of the pregnant patients	24.03	24.7	0.60
Primigravidity	37 (68.50)	73 (45.10)	<0.001
Chorioamnionitis*	4 (7.40)	2 (1.20)	0.01
Maternal morbidity	20 (37.70)	50 (32.60)	0.73
Use of oxytocin	17 (32.70)	130 (80.20)	<0.001
Non-monitoring fetal heart rate	13 (24.10)	3 (1.90)	<0.001
Non-monitoring of labor	16 (29.60)	3 (1.90)	<0.001
Meconium amniotic fluid	33 (62.30)	21 (13.50)	<0.001
Gestational age: <38 weeks	17 (31.50)	48 (29.60)	0.80
Sex:			
Female	22 (40.70)	73 (45.10)	0.57
Male	32 (59.30)	89 (54.90)	
Cesarean section	20 (37.70)	67 (41.60)	0.61
Neonatal mortality*	3 (5.60)	0 (0.00)	<0.001
Alterations of the fetal heart rate: yes*	10 (24.40)	1 (0.60)	<0.001
Alterations of labor monitoring: yes	20 (51.28)	14 (8.80)	<0.001

Cali, Colombia 2012–2014.
The general characteristics of the pregnancy and newborn in relation to the biological variables are described, according to case or control.
**Chi-square test was performed for variables with frequencies >5, and Fisher's exact-test was used in the opposite case.*
***Significance level of 0.05.*

TABLE 2 | Description of social variables.

Variable	Cases (54) (%)	Control (162) (%)	*P*-value
Maternal schooling: high school	26 (48.2.0)	124 (76.50)	<0.001
Without a partner	17 (32.70)	42 (25.90)	0.34
Household income: less than the minimum wage	35 (79.50)	84 (54.50)	0.09
Prenatal control: ≤3	22 (41.50)	26 (16.10)	<0.001
Unplanned pregnancy	30 (68.20)	59 (38.60)	<0.001
MOS survey			
Emotional subscale: poor support*	18 (33.30)	27 (16.70)	<0.001
Social subscale: poor support**	12 (22.60)	1 (0.60)	<0.001
Affective subscale: poor support***	20 (37.70)	19 (11.70)	<0.001
Instrumental subscale: poor support****	23 (42.60)	23 (14.20)	<0.001

Cali, Colombia 2012–2014.
Significance level of 0.05.
**Emotional subscale: low support < 24 points.*
***Social subscale: low support < 9 points.*
****Affective subscale: low support < 9 points.*
*****Instrumental subscale: low support < 12 points.*

bivariate analysis but were not significant when entering the final model.

DISCUSSION

The objective of this study was to determine if a relationship existed between psychosocial and biological factors with the development of perinatal asphyxia, which has been demonstrated. The psychosocial and biological factors remained significant after adjusting for different variables. Education higher than primary basic school had a protective effect; on the other hand, low antenatal control and schooling were associated with greater odds for perinatal asphyxia. These results show how social disadvantages were associated with greater risks; their assessment and surveillance in disadvantaged populations may eventually help to reduce current disparities and improve pregnancy outcomes across the socioeconomic spectrum. These results support the previous research published by Ellis et al. (20).

The psychosocial factors have an important impact on the fetal outcome, as proposed by Kramer et al. (21) in a study that identifies the poorest areas as those with the greatest difficulty in access to health services, which reflects one of the dimensions

TABLE 3 | Bivariate analysis between perinatal asphyxia and biological variables.

Variable	Cases (54) %	Controls (162) %	*P*-value***	OR 95% CI
Multiparity*	17 (31.48)	89 (54.94)	<0.001	0.37 (0.19–0.72)
Chorioamnionitis	4 (7.41)	2 (1.23)	0.04	5.95 (1.05–33.44)
Preeclampsia and eclampsia	20 (37.04)	26 (16.25)	<0.001	3.04 (1.51–6.12)
Maternal morbidity**	20 (37.74)	50 (32.68)	0.52	1.22 (0.64–2.35)
Use of oxytocin	17 (32.69)	130 (80.25)	<0.001	0.12 (0.06–0.24)
FCF monitoring	41 (75.93)	159 (98.15)	<0.001	0.06 (0.01–0.22)
Monitoring of labor	38 (70.37)	159 (98.15)	<0.001	0.04 (0.01–0.17)
Induction of labor	14 (25.93)	131 (82.39)	<0.001	0.07 (0.03–0.15)
Meconium amniotic fluid	33 (62.26)	21 (13.46)	<0.001	11.71 (5.46–25.14)
Alterations of fetal heart rate	10 (24.39)	1 (0.62)	<0.001	51.93 (6.41–420.4)

Cali, Colombia 2012–2014.
**In this study, multiparity is defined as having more than one delivery.*
***Maternal morbidity: includes diabetes, hypertension, preeclampsia, and eclampsia.*
****Significance level of 0.05.*

TABLE 4 | Bivariate analysis between perinatal asphyxia and social variables.

Variable	Cases (54) %	Controls (162) %	*P*-Value*	OR 95% CI
Schooling: high school	26 (48.15)	124 (76.54)	<0.001	0.32 (0.17–0.60)
With partner	35 (67.31)	120 (74.07)	0.36	0.73 (0.37–1.43)
1–2 legal minimum wage	9 (20.45)	70 (45.45)	<0.001	0.30 (0.13–0.68)
Antenatal visits ≥4	30 (58.82)	136 (85.00)	<0.001	0.25 (0.12–0.51)
MOS survey				
Emotional subscale poor support[a]	18 (33.33)	27 (16.70)	0.01	2.51 (1.25–5.06)
Social subscale poor support[b]	12 (22.20)	1 (0.62)	<0.001	53.55 (6.66–430.05)
Affective subscale poor support[c]	20 (37.0)	19 (11.70)	<0.001	4.37 (2.08–9.20)
Instrumental subscale poor support[d]	23 (42.59)	23 (14.20)	<0.001	4.17 (2.07–8.38)
Total classification poor support	22 (40.70)	26 (16.10)	<0.001	3.34 (1.67–6.65)

Cali, Colombia 2012–2014.
**Significance level of 0.05.*
[a]Emotional subscale: low support < 24 points.
[b]Social subscale: low support < 9 points.
[c]Affective subscale: low support < 9 points.
[d]Instrumental subscale: low support < 12 points.

of social exclusion. It is proposed, "that expansion of access and quality of care can have a positive impact on reducing early neonatal mortality." Therefore, social support has been considered "part of social relationships and refers to the help or assistance provided through interpersonal transactions. It is subdivided into instrumental (provision of tangible help and services), informative (provision of information, advice, or useful suggestions to solve problems), evaluative (provision of useful information for self-evaluation), and emotional (expression of empathy, love, trust, and concern). Social support nurtures the individual from its closest nucleus, which is the family, although there are also sources of external support, such as friends, community, and other actors" (18). The present study evaluated the social support in pregnant women for the development of perinatal asphyxia by using the MOS questionnaire. It was identified that poor social support (inadequate instrumental support) in pregnant women led to a six times greater opportunity of developing asphyxia. Different disciplines have hypothesized that psychosocial factors are related to morbidity and mortality, as in cardiovascular diseases, cancer, and premature birth (22–24).

The biological variables in the final model that were found to be significantly associated with the moderate or severe perinatal asphyxia event were the presence of meconium amniotic fluid, which was associated with a higher odds of asphyxia that is 15 times in comparison to those without it, and the induction of labor and monitoring of the fetal heart rate, which lowered the odds. The presence of meconium in amniotic fluid at the time of delivery has been reported in other investigations; a case–control study in Sweden (25) showed that having meconium amniotic fluid increased by four-fold the development of perinatal asphyxia. In a systemic review, McIntyre et al. (26) reported how the presence of meconium-stained fluid and especially meconium aspiration were strong

TABLE 5 | Multivariate analysis.

Variable	OR	Adjusted OR	*P*-value **	95% CI
Meconium amniotic fluid	11.71	15.28	0.02	2.78–83.94
Induction of labor	0.07	0.03	<0.001	0.01–0.21
Monitoring of fetal heart rate	0.06	0.01	0.01	0.00–0.31
Secondary education	0.32	0.15	0.02	0.03–0.77
Instrumental subscale*	4.17	6.44	0.03	1.16–35.66
Prenatal visits	0.25	0.25	0.13	0.04–1.50

Perinatal asphyxia with relation to biological and social factors. Cali, Colombia 2012–2014.
Multivariate analysis after adjusting the model.
**Instrumental subscale: low support < 12 points.*
***Significance level of 0.05.*

factors associated with neurological compromise at all gestational ages. The presence of meconium in the amniotic fluid can be part of normal processes or followed by abnormal situations, such as compression of the umbilical cord or uteroplacental insufficiency, considered indicators of fetal distress. Meconium is not always associated with intrapartum problems, morbidity, or neonatal mortality (27). Adverse outcomes have been observed when related to other signs of fetal intolerance at delivery, like late decelerations, increased fetal heart rate, and decreased beat-to-beat variability evaluated through continuous electronic cardiac fetal monitoring. The presence of meconium amniotic fluid is considered a controversial factor, and its importance is permanently a subject of analysis. It is discussed if it can behave as a marker of fetal non-well-being or a direct factor of greater damage (28, 29).

Alterations observed during the monitoring of labor have been shown to be a factor significantly associated with the presence of perinatal asphyxia (25). Without adequate control of the fetal heart rate and monitoring of labor, it is not possible to diagnose high-risk events in time, such as pre-mature placental abruption, meconium fluid, and unsatisfactory fetal status (30). The omission of a partograph during labor was also found to be a factor significantly associated with the presence of perinatal asphyxia (OR 2.25, 95%CI 1.14–4.45). As indicated in a previous study in 2011 (16), in this same center, without proper control and monitoring of labor with an appropriate partograph, it is not possible to diagnose in time two main adverse outcomes such as pre-mature placental abruption and labor with a prolonged expulsive phase. Evidence of an altered fetal heart rate during labor, such as severe and sustained bradycardia, absence of variability, and persistent or tardive decelerations, has been shown to increase up to eight times the likelihood of neurological alterations and asphyxia in newborns (22).

The predictive power of electronic fetal monitoring for the development of neonatal encephalopathy and cerebral palsy is low, and, to date, the use of this monitoring has not decreased the incidence of cerebral palsy (30). Despite this, electronic fetal monitoring is the best tool that obstetricians can use in labor to identify fetal metabolic acidosis and reduce the probability of death or neonatal seizures during labor and delivery (31).

The protective effect of inducing labor, at 97% evidenced in this study, is probably related to the fact that women requiring this management are referred from other centers of less complexity, with a diagnosis of fetal non-well-being, and in conditions that require urgent cesarean section. A similar situation occurs for monitoring of the fetal heart rate that showed a protection of 99% when performed. These factors showed the probability of preventing the event if conditions of adequate and timely access to health services are guaranteed. That is why it is considered particularly important to evaluate the quality of health services, emphasizing on how investing in qualified human resources, as well as physical and technological infrastructure conditions, guarantees and provides adequate care to the mother and child in all levels of care. Thus, these events that result in disability and death can be minimized. With these results, it is possible to state that social and biological conditions in vulnerable populations, such as the one cared for in the center where the study was conducted, have a higher odds of developing perinatal asphyxia due to poor support in the instrumental subscale (MOS survey), low schooling, presence of meconium in the amniotic fluid, lack of labor induction, and inadequate fetal heart rate monitoring.

This study shows the importance of studying perinatal asphyxia as a potential public health problem. It also allows understanding that interventions in biopsychosocial factors, associated with the development of perinatal asphyxia, favor its reduction, particularly in middle- and low-income populations. In our study, maternal education level associated with moderate or severe perinatal asphyxia when pregnant women did not have a primary education. This factor is recognized in other publications as a condition associated with maternal and neonatal morbidity and mortality in low-income countries (32, 33).

Findings of the present study support previous research on the association of meconium amniotic fluid with perinatal asphyxia (2, 25, 26, 28), which, together with perinatal acidosis and alterations in the monitoring of labor, may help clinicians to identify women that require early obstetric interventions. Induction of labor and monitoring of the fetal heart rate, in this research, were associated as factors with a protective effect; the lack of monitoring of maternal and fetal conditions at labor was probably related to the lack of appropriate healthcare infrastructure, equipment, and trained clinicians.

Therefore, pregnant women are referred to higher complexity-level institutions where they enter in critical conditions with emergency cesarean requirements or in the final phase of labor, which has repercussions on the health of the newborns.

To achieve the required changes in mortality outcomes and sequelae of this disease, participation from different disciplines and sectors committed to its early identification is required; thus, it is necessary to clearly define groups at risk. According to the results of this study, pregnant women with social risk factors, such as low schooling, inadequate prenatal care, and low instrumental social support, should be included as high priority in the guidelines of our health system in order to improve the quality of their prenatal care.

Regarding the limitations of the study, one of them was the fact of it being retrospective, which favors the presentation of bias, such as memory, during the collection of information in the MOS survey. Another possible bias was the selection of participants, given that most of the population was of low income; hence, they were more likely to be exposed to adverse social and biological conditions, although in the end, no significant differences were demonstrated between the cases and the controls according to household income: less than the minimum wage ($p < 0.09$). Incomplete medical records correspond to 7.3%, which did not significantly affect the analysis.

The strengths of this work highlight the contribution of the validated MOS questionnaire in the results of social variables.

In conclusion, these findings confirm the hypothesis that perinatal asphyxia is related to social and biological factors susceptible to intervention through strategies involving adequate prenatal visits and delivery care. Studies should continue to identify risk factors applicable to our population and early markers of asphyxia that may influence its presentation.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the University Hospital del Valle. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

JT-M: design, analysis, and writing of the article. JF-P: analysis and writing of the article. KL: collection of information and writing of the article. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Hyder A, Wali S, McGuckin J. The burden of disease from neonatal mortality: a review of South Asia and Sub-Saharan Africa. *Int J Obstet Gynaecol*. (2003) 110:894–901. doi: 10.1111/j.1471-0528.2003.02446.x
2. Moss W, Darmstadt G, Marsh D, Black R, Santosham M, Research priorities for the reduction of perinatal and neonatal morbidity and mortality in developing country communities. *J Perinatol*. (2002) 22:484–95. doi: 10.1038/sj.jp.7210743
3. Peter JM. The etiology and pharmacology approach to hypoxic-ischemic encephalopathy in the newborn. *Neo Rev*. (2002) 3:e99–107. doi: 10.1542/neo.3-6-e99
4. Lawn JE, Cousens S, Zupan J. Four-million neonatal deaths: when? where? why. *Lancet*. (2005) 365:891–900. doi: 10.1016/S0140-6736(05)71048-5
5. World Health Organization. *Basic Newborn Resuscitation; A Practical Guide*. (2007). Available online at: https://www.who.int/reproductivehealth/publications/en/ (accessed January 2015).
6. Shannon M. Clark, Sanmaan K. Basraon and Gary D.V. Hankins. Intrapartum asphyxia, neonatal encephalopathy. Cerebral palsy and obstetric interventions in the term and near-term infant. *Neo Rev*. (2013) 14:e13–21. doi: 10.1542/neo.14-1-e13
7. Vannucci, RC, Perlman, JM. Interventions for perinatal hypoxic ischemic encephalopathy. *Pediatrics*. (1997) 100:1004–14. doi: 10.1542/peds.100.6.1004
8. Palmer C, Vannucci RC. Potential new therapies for perinatal cerebral hypoxia-ischemia. *Clin Perinatol*. (1993) 20:411–27. doi: 10.1016/S0095-5108(18)30401-9
9. Pshiirrer, ER. Yeumans. E. Does asphyxia cause cerebral palsy? *Sem Perinatol*. (2000) 24:215–20. doi: 10.1053/sper.2000.7080
10. Phibbs R, Sola A, Rogido M. Asfixia. In: Sola A, Rogido M, editors. *Cuidados especiales del feto y el recién nacido*. Buenos Aires, Sola A: Interamericana. (2001). p. 95–1022.
11. Centro Latinoamericano de Perinatología y Desarrollo Humano (CLAP). *Estadísticas de la Región de América Latina y Caribe*. (2005). Available online at: https://www.paho.org/es/centro-latinoamericano-perinatologia-salud-mujer-reproductiva-clap (accessed January 2015).
12. Jaramillo MC, Varela A, Gallego AI. Cumplimiento de los objetivos de desarrollo del milenio en Santiago de Cali. In: Villegas AV, editor. *Evaluación y seguimiento. Secretaría de Salud Pública Municipal de Cali-CIENFI*. Cali: Feriva, Universidad ICESI (2010). p. 96–121.
13. Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress. A clinical and electroencephalographic study. *Arch Neurol*. (1976) 33:696–705. doi: 10.1001/archneur.1976.00500100030012
14. Ballard JL, Khoury JC, Wedig K, Wang L, Eilers-Walsman BL, Lipp R. New Ballard Score, expanded to include extremely premature infants. *J Pediatr*. (1991) 119:417–23. doi: 10.1016/S0022-3476(05)82056-6
15. Van Handel M, Swaab H, Vries L, Jongmans. M. Long-term cognitive and behavioral consequences of neonatal encephalopathy following perinatal asphyxia: a review. *Eur J Pediatr*. (2007) 166:645–54. doi: 10.1007/s00431-007-0437-8
16. Torres-Muñoz J, Rojas C, Mendoza-Urbano D, Marín-Cuero D, Orobio S, Echandía C. Risk factors associated with the development of perinatal

asphyxia in neonates at Hospital Universitario del Valle, Cali, Colombia, 2010-2011. *Biomédica*. (2017) 37(Suppl. 1):51–6. doi: 10.7705/biomedica.v37i1.2844
17. Connor RJ. Sample size for testing differences in proportions for the paired-sample design. *Biometrics*. (1987) 43:207–11. doi: 10.2307/2531961
18. Arredondo NHL, Rogers HL, Tang JFC, Gómez SLP, Ariza NLO, Pérez MAJ, et al. Validation of the Colombian MOS social support survey. *Int J Psychol Res*. (2012) 5:142–50. doi: 10.21500/20112084.770
19. Sherbourne CD, Stewart AL. The MOS social support survey. *Soc Sci Med*. (1991) 32:705–14. doi: 10.1016/0277-9536(91)90150-B
20. Ellis M, Manandhar N, Manandhar DS, Costello A. Risk factors for neonatal encephalopathy in Kathmandu, Nepal, a developing country: unmatched casecontrol study. *BMJ*. (2000) 320:1229. doi: 10.1136/bmj.320.7244.1229
21. Kramer MS, Goulet L, Lydon J, Séguin L, McNamara H, Dassa C, et al. Socio-economic disparities in preterm birth: causal pathways and mechanisms. *Paediatr Perinat Epidemiol*. (2001) 15:194–210. doi: 10.1046/j.1365-3016.2000.00266.x
22. Maisonneuve E, Audibert F, Guilbaud L, Lathelize J, Jousse M, Pierre F, et al. Risk factors for severe neonatal acidosis. *Obstetr Gynecol*. (2011) 118:818–23. doi: 10.1097/AOG.0b013e31822c9198
23. Daripa M, Caldas HMG, Flores LPO, Waldvogel BC, Guinsburg R, Almeida MFBD. Perinatal asphyxia associated with early neonatal mortality: populational study of avoidable deaths. *Revista Paulista de Pediatria*. (2013) 31:37–45. doi: 10.1590/S0103-05822013000100007
24. Heaman MI, Blanchard JF, Gupton AL, Moffatt ME, Currie RF. Risk factors for spontaneous preterm birth among Aboriginal and non-Aboriginal women in Manitoba. Paediatr Perinatal Epidemiol. (2005) 19:181–93. doi: 10.1111/j.1365-3016.2005.00644.x
25. Milsom I, Ladfors L, Thiringer K, Niklasson A, Thornberg E. Influence of maternal, obstetric and fetal risk factors on the prevalence of birth asphyxia at term in a Swedish urban population. *Acta Obstet Gynecol Scand*. (2002) 81:909–17. doi: 10.1080/j.1600-0412.2002.811003.x
26. McIntyre S, Taitz D, Keogh J, Goldsmith S, Badawi N, Blair EVE. A systematic review of risk factors for cerebral palsy in children born at term in developed countries. *Dev Med Child Neurol*. (2013) 55:499–508. doi: 10.1111/dmcn.12017
27. Lavery JP. Meconium in the amniotic fluid: a risk manager's perspective. *J Healthcare Risk Manag*. (2004) 24:21–6. doi: 10.1002/jhrm.5600240405
28. Kariniemi V, Harrela M. Significance of meconium staining of the amniotic fluid. *J Perinat Med*. (1990) 18:345–9. doi: 10.1515/jpme.1990.18.5.345
29. Tolu LB, Birara M, Teshome T, Feyissa GT. Perinatal outcome of meconium stained amniotic fluid among labouring mothers at teaching referral hospital in urban Ethiopia. *PLoS ONE*. (2020) 15:e0242025. doi: 10.1371/journal.pone.0242025
30. Clark SM, Ghulmiyyah LM, Hankins, GD. Antenatal antecedents and the impact of obstetric care in the etiology of cerebral palsy. *Clin Obstetr Gynecol*. (2008) 51:775–86. doi: 10.1097/GRF.0b013e3181870994
31. Arnold KC, Flint CJ. Intrapartum fetal heart rate monitoring: nomenclature, interpretation, and general management principles. *Obstet Essent*. (2017) 27:101–7. doi: 10.1007/978-3-319-57675-6_16
32. Herrera JA, Hurtado H, Cáceres D. Antepartum biopsychosocial risk and perinatal outcome. *Fam Pract Res J*. (1992) 12:391–9.
33. Herrera JA, Salmerón B, Hurtado H. The biopsychosocial risk assessment and the low birthweight. *Soc Sci Med*. (1997) 8:1107–14. doi: 10.1016/S0277-9536(96)00240-7

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