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The Prenatal High Risk Factors in Non-Syndromic Cleft Lip or/and Palate Fetuses: A Cohort Study Based on Maternal Health Care Records of a Population

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Abstract

Background/Aim: To determine the associations of different prenatal factors with the development of non-syndromic cleft lip or/and palate (NSCL/P), as there are still no consensus to the risk factors of NSCL/P.

Methods: Nested case-control study based on the gestational period health care record data of all singleton NSCL/P (n=197) and fetuses without birth defects (n=192706) born in-hospital of Zhongshan between 2016 and 2019.

Results: Vagina infected (OR 3.43, 95% CI 1.34-7.19), assisted conception (OR 11.57, 95% CI 0.65-54.29), folic acid intake <3 months during pre- and early pregnancy (OR 1.57, 95% CI 1.10-2.30), incomplete placenta or rough surface placenta (OR 4.65, 95% CI 3.23-6.57), abnormal amniotic fluid (OR 2.74, 95% CI 2.04, 3.67), low birth weight of newborns (OR 5.44, 95% CI 3.41-8.54), preterm delivery (OR 3.29, 95% CI 2.07-5.24), and post-term delivery (OR 5.94, 95% CI 0.97-18.93) were associated with a higher risk of NSCL/P.

Conclusions: The status of placenta and fluid were discovered as risk factors with NSCL/P, which strengthens the importance role of interaction between the placental barrier and various environmental factors on the development of NSCL/P.

Keywords: Cleft lip or/and palate; Prenatal risk factors; Maternal health care records; Cohort study

Abbreviations: NSCL/P: Non-Syndromic Cleft Lip or/and Palate; CL/P: Cleft Lip or/and Palate; BMI: Body Mass Index; HBsAg: Hepatitis B Surface Antigen; G6PD: Glucose-6-Phosphate Dehydrogenase Deficiency; MLRM: Multiple Logistic Regression Model; VIF: Variance Expansion Factor; CIs: Confidence Intervals

Introduction

Cleft lip or/and palate (CL/P) are most common serial congenital anomalies to affect the orofacial region, and the overall incidence of CL/P is approximately 1 in every 700 to 1000 births with wide variation across geographic areas, ethnic group and nature of cleft itself [1-3]. CL/P occurs between the 6th and 12th week of pregnancy during early embryologic development, and has complex etiology thought to involve genetic influences with variable interactions from environmental factors [4-6].

Non-syndromic cleft lip or/and palate (NSCL/P) accounts for 70% of CL/P cases, and it is isolated anomaly without other congenital anomalies. Several previous studies have reported more than 30 genetic risk loci that are responsible for pathogenesis of NSCL/P, but the major cause of NSCL/P has not been fully elucidated due to complex interaction between genetic and environmental risk factors [7,8]. Meanwhile, the reported environmental factors associates with NSCL/P are variational in different studies, and there was few study focus on prenatal factors of NSCL/P. However, the prenatal physiologic and pathological changes reflect the effects of environmental factors on fetuses, further large sample sizes study on prenatal factors of NSCL/P is needed.

The aim of this study was to determine the associations of different prenatal factors with the development of NSCL/P. The gestational period health care record data of 192903 fetuses born between 2016 and 2019 in one city region were used to explore the prenatal risk factors on NSCL/P.

Methods

Study design and participants

This nested case-control study included 192903 singleton fetuses were born (included artificial termination of death pregnancy) in-hospital from January 1, 2016 to December 31, 2019 in Zhongshan City of China. We extracted the maternal health care record data of total 204119 pregnant women and their 206850 fetuses from the regional information system databases of maternal and children health care of Zhongshan during this period, and all the private identity information was excluded when we extracted the data. All the target fetuses delivered between 2016 and 2019, we excluded 5446 twins and multiple pregnancies, 23 singleton fetuses with syndromic cleft lip or/and palate (SCL/P), and 8478 singleton fetuses with other birth defects (no cleft lip or/and palate), and then there were 192903 singleton fetuses were included in the final pregnancy cohort. In the final cohort, the cases were 197 singleton fetuses with non-syndromic cleft lip or/and palate (NSCL/P), the controls were 192706 singleton fetuses without NSCL/P and without other birth defects.

We confirm that all methods were carried out in accordance with relevant guidelines and regulations of ethical review of biomedical research involving human beings issued by Health and Family Planning Commission of the People's Republic of China in 2016. This study used the de-identified historical health record data belong to information centre of health bureau of Zhongshan city, and informed consent was waived by the ethical review board of Guangdong Women and Children Hospital.

From the real records data, we got these variables in prenatal period as candidate risk factors, included mother's age, education level, job, per capita household income, pre-pregnancy body mass index (BMI), folic acid intake during pre- and early pregnancy, risk factors exposure in early pregnancy, illness history, assisted conception, prenatal Down's screening, liver function test, renal function test, urine occult blood, hepatitis B surface antigen (HBsAg), syphilis test, scarred uterus, gestational diabetes mellitus, hypertensive disorder, thalassemia carrier, anemia, thyroid dysfunction or abnormalities, vagina infected, glucose-6-phosphate dehydrogenase deficiency (G6PD), incomplete placenta, placental roughness, torsion of umbilical cord, umbilical cord around neck, amniotic fluid abnormal, fetal position, and gender of newborns.

Statistical analysis

The data were analysed using R version 3.6.2 (2019-12-12). Firstly, all variables were tested by χ^2 tests. Secondly, all variables were used to set-up a multiple logistic regression model (MLRM), and variance expansion factor (VIF) scores were calculated to verify the multicollinearity of independent variables. The variables of illness history and HBsAg were excluded from the MLRM for their VIF scores >10, and a new MLRM was set-up. And then a final simple MLRM was set-up using the variables with the p value <0.05 in the new MLRM, the ORs and their 95% confidence intervals (CIs) of these variables were calculated. The missing values of variables were recoded to unknown for analysis. In all analyses, p values <0.05 were considered significant.

Results

General characters

In all 192903 fetuses, there were 197 (0.10 %) non-syndromic cleft lip or/and palate (NSCL/P), among them, there were 74 (37.56%) cleft lips without palates, 43 (21.83%) cleft palates without lips, and 80 (40.61%) cleft lips with palates. The prenatal general characteristics of all fetuses and their mothers are given in table 1. By χ^2 tests, it showed education of mothers, pre-pregnancy BMI grades of mothers, and folic acid intake during pre- and early pregnancy might associate with NSCP.

Prenatal risk factors

The prenatal risk factors of all fetuses and their mothers are given in table 2. By χ^2 tests, it showed risk factors exposure in early pregnancy, vagina infected, incomplete placenta, rough placenta, abnormal amniotic fluid, illness history of mothers, and prenatal down's screening might associated with NSCP.

Of the 197 NSCL/P, 183 cases (92.89%) had unilateral NSCL/P, and 14 cases (7.11%) had bilateral NSCL/P. Table 3 showed the factors are associated with unilateral NSCL/P and which ones are associated with bilateral NSCL/P. By χ^2 tests, all the factors with p value less than 0.05 were listed.

The results of the final simple logistic regression model were given in table 4. Risk factors exposure in early pregnancy, scarred uterus, vagina infected, assisted conception, incomplete placenta, placental roughness, abnormal amniotic fluid, not head presentation, folic acid intake <3 bottles during pre- and early pregnancy, mother's education is primary school or no schooling, and high risk of prenatal Down's screening were confirmed as the prenatal high risk factors on NSC/P.

Discussion

Orofacial clefts particularly cleft lip or/and cleft palate (CL/P) being a major public health problem, affecting thousands of children worldwide each year, with a frequency of 1 in 500 affecting more than 2.6 million people in China [7,9]. Many researches have focused on NSCL/P, especially with a target to distinguish the underlying genetic risk loci behind pathogenicity of CL/P, but as they all together accounts for only 20%-25% of NSCL/P heritability. 7 Most cases of CL/P are diagnosed in utero by ultrasound after 20 week's gestation, but some are undetected and only discovered at delivery and a small percentage of clefts are diagnosed during childhood [10,11]. So it is important to confirm the prenatal environmental and biological risk factors for auxiliary early diagnosis and etiology on NSCL/P.

The previous researches are still no consensus exists as to the cause of NSCL/P, researchers generally agree that genetic, nutritional, and environmental factors contribute to their formation [12].

Various reported environmental factors include smoking, alcohol use, some medications, folate deficiency, maternal obesity, maternal diseases such as diabetes and stress during pregnancy, chemical exposure, smoke from cooking indoors exposure [1,12-21]. But we found the risk factors exposure in early pregnancy had no association with NSCL/P when we took into account extensive prenatal factors, these factors include fever and other sicks, take medicines, radiation exposure, smoke, or drink wine, etc., however, each of these factors were seldom in pregnant couples for the popular practice of health care before pregnancy among the mothers of this study, so we took into account these risk factors together. It was different from some previous reports, this study showed maternal BMI, higher level of maternal education, family history of clefts, high or low parental age at time of childbirth, and maternal diseases such as diabetes were not associated with NSCL/P [1,22-24].

This study reflects only more than 3 months of daily intake of 0.4 mg folic acid before and during early pregnancy may help to reduce the risk of NSCL/P. However, there was a high heterogeneity between

Table 1: The prenatal general characters of 192903 pregnant women and their fetuses without and with NSCP[a]

General Characters	No NSCL/P (n=192706)	NSCL/P(n=197)	P-value
Age grades of mothers			0.059
<20 years	6939 (3.60%)	10 (5.08%)	
20-35 years	160812 (83.45%)	152 (77.16%)	
≥ 35 years	24955 (12.95%)	35 (17.77%)	
Years of mother's education			0.24
≤ 12 years	118799 (61.65)	130 (65.99)	
>12 years	73907 (38.35)	67 (34.01)	
Job of mothers			0.89
Temporary and unemployed	15836 (8.22%)	14 (7.11%)	
Housework	41351 (21.46%)	43 (21.83%)	
Service staff	9783 (5.08%)	10 (5.08%)	
Worker	25129 (13.04%)	33 (16.75%)	
Enterprises and sale staff	25721 (13.35%)	25 (12.69%)	
Technical and public service	15254 (7.92%)	15 (7.61%)	
Others	18152 (9.42%)	19 (9.64%)	
Unknown	41480 (21.53%)	38 (19.29%)	
Per capita household income grades			0.099
>8000 RMB	28355 (14.71%)	40 (20.30%)	
<2000 RMB	1585 (0.82%)	3 (1.52%)	
2000-8000 RMB	71902 (37.31%)	68 (34.52%)	
Unknown	90864 (47.15%)	86 (43.65%)	
Pre-pregnancy BMI grades of mothers[b]			0.018
<18.5	29873 (15.50%)	20 (10.15%)	
18.5-25	103541 (53.73%)	101 (51.27%)	
>25	14130 (7.33%)	13 (6.60%)	
Unknown	45162 (23.44%)	63 (31.98%)	
Folic acid intake during pre- and early pregnancy[c]			0.031
≥ 3 bottles (months)	47465 (24.63%)	35 (17.77%)	
<3 bottles (months)	145241 (75.37%)	162 (82.23%)	
Gender of newborns			0.47
Female	91551 (47.51%)	85 (43.15%)	
Male	101155 (52.49%)	112 (56.85%)	

[a]NSCL/P is non-syndromic cleft lip or/and palate.
[b]BMI is body mass index.
[c]There are 31 tablets per bottle, and 0.4 mg folic acid per tablet, the recommended daily intake is one tablet in 3 months before pregnancy and in first 3 months of pregnancy for every pregnant woman.

the previous studies on the role of folate in the etiology of CL/P, such as population characteristics, variation in timing of exposure and supplement types, and publication bias. A previous study also showed low consumption of folic acid was found to be a risk factor of CL/P [22]. A review has reported that high-dose folic acid probably has a role in prevention of recurrence of isolated CL/P in high-risk individuals, but not cleft palate [25]. Another review has reported that mandatory folic acid fortification of wheat and/or maize flour may have beneficial effects on non-syndromic CL/P [26]. A recent meta-analysis also has shown that the risk of non-syndromic orofacial clefts was reduced among pregnant women with folic acid-containing supplements during the etiologically relevant period [27]. In Zhongshan of China, every pregnant woman is recommended daily intake of 0.4 mg folic acid in 3 months before pregnancy and in first 3 months of pregnancy. This study also showed that less than 3 months of daily intake of 0.4 mg folic acid during pre- and early pregnancy was associated with the development of NSCL/P.

This study revealed that vagina infected fungi, bacteria, trichomonad, or gonorrhea as a total may increase the risk of NSC/LP. A previous report has shown maternal self-reported genital tract infections were associated with CL/P, but the strength of that conclusion was limited [28]. Meanwhile, in our study, the number of vagina infected (only 6 of 197 NSCL/P) was small and the occurrence of infected timing couldn't be determined in early pregnancy, so further study is still needed to confirm the association between vagina infected and NSCL/P.

We found assisted conception was associated with NSCL/P. Fauque P, et al. have reported a moderately increased risk of defects (i.e. CL/P, etc. the 15 relevant subgroups of malformations) subsisted after IVF in a large study [29]. A meta-Analysis has reported that the IVF/ICSI singleton pregnancies were significantly associated with high birth prevalence of congenital malformations (included CL/P), but it remains uncertain whether detected differences represent true or methodological differences [30]. However, this study only had a small number of assisted conception pregnancies (100 of 192706 no NSCL/P *vs.* 1 of 197 NSCL/P), so the strength was limited.

We found several new prenatal factors increased the risk of the development of NSCL/P, they were incomplete placenta or rough surface placenta, abnormal amniotic fluid, and preterm or post-term delivery of newborns. Similar to the report, we also found low birth weight of newborns was associated with NSCL/P [24]. According to the results of this study, placental barrier may play a key role in the effect of environmental factor on NSCL/P, and a well status of placenta decreases the adverse effects of prenatal risk factors exposure. Meanwhile, maybe there are common risk factors between NSCL/P and preterm and low birth weight.

There are, however, several limitations. Firstly, we couldn't include all the previous reported risk factors of NSCL/P in this study, because we used the existing health care record databases to analyze. Secondly, although there was a large number in this retrospect cohort, but the number of NSCL/P was small (197 NSCL/P *vs.* 192706 no NSCL/P). Several risk factors showed in this study are needed further study to confirm.

Conclusion

Vagina infected, assisted conception, and insufficiency folic acid intake before and during early pregnancy, low birth weight of newborns were associated with a higher risk of the development of NSCL/P, which similar to previous studies. Our study adds several new prenatal risk factors of NSCL/P, includes incomplete placenta or rough surface placenta, abnormal amniotic fluid, preterm delivery

Table 2: The prenatal risk factors of 192903 pregnant women and their fetuses without and with NSCP[a]

Prenatal Risk Factors	No NSCL/P (n=192706)	NSCL/P (n=197)	P-value
Risk factors exposure in early pregnancy[b]	5733 (2.97%)	14 (7.11%)	0.0014
Assisted conception	100 (0.05%)	1 (0.51%)	0.22
Scarred uterus	20930 (10.86%)	27 (13.71%)	0.24
Gestational diabetes mellitus	15773 (8.19%)	15 (7.61%)	0.87
Hypertensive disorder	5068 (2.63%)	8 (4.06%)	0.3
Thalassemia carrier	3869 (2.01%)	7 (3.55%)	0.2
Anemia	7650 (3.97%)	10 (5.08%)	0.54
Thyroid dysfunction or abnormalities	1372 (0.71%)	3 (1.52%)	0.35
Vagina infected[c]	1855 (0.96%)	6 (3.05%)	0.0087
G6PD[d]	996 (0.52%)	3 (1.52%)	0.14
Incomplete placenta or rough surface placenta	6567 (3.41)	46 (23.35)	0
Torsion of umbilical cord	1994 (1.03%)	2 (1.02%)	1
Umbilical cord around neck	52535 (27.26%)	46 (23.35%)	0.25
Abnormal amniotic fluid[e]	32258 (16.74%)	76 (38.58%)	0
Not head presentation	15092 (7.83%)	24 (12.18%)	0.032
Low birth weight of newborns	8146 (4.23)	78 (39.59)	0
Illness history of mothers			0
No	187173 (97.13%)	182 (92.39%)	
Yes	3022 (1.57%)	4 (2.03%)	
Unknown	2511 (1.30%)	11 (5.58%)	
Prenatal Down's screening			0.031
Low risk	120237 (62.39%)	105 (53.30%)	
High risk	67866 (35.22%)	86 (43.65%)	
Unknown	4603 (2.39%)	6 (3.05%)	
Liver function test[f]			0.16
Normal	60148 (31.21%)	49 (24.87%)	
Abnormal	859 (0.45%)	1 (0.51%)	
Unknown	131699 (68.34%)	147 (74.62%)	
Renal function test[g]			0.14
Normal	58562 (30.39%)	47 (23.86%)	
Abnormal	980 (0.51%)	1 (0.51%)	
Unknown	133164 (69.10%)	149 (75.63%)	
Urine occult blood			0.95
Negative	14811 (7.69%)	15 (7.61%)	
Positive	1360 (0.71%)	1 (0.51%)	
Unknown	176535 (91.61%)	181 (91.88%)	
Hepatitis B surface antigen			0.13
Negative	154760 (80.31%)	150 (76.14%)	
Positive	12132 (6.30%)	11 (5.58%)	
Unknown	25814 (13.40%)	36 (18.27%)	
Syphilis test			0.15
Negative	165494 (85.88%)	160 (81.22%)	
Positive	453 (0.24%)	1 (0.51%)	
Unknown	26759 (13.89%)	36 (18.27%)	
Delivery period of newborns			0
Preterm delivery	9268 (4.81)	74 (37.56)	
Term delivery	183002 (94.96)	121 (61.42)	
Post-term delivery	436 (0.23)	2 (1.02)	

[a]NSCL/P is non-syndromic cleft lip or/and palate.
[b]Risk factors include fever, other sickness, take medicines, radiation exposure, smoke, or drink wine, etc.
[c]Vagina infected fungi, bacteria, trichomonad, or gonorrhea.
[d]G6PD is glucose-6-phosphate dehydrogenase deficiency.
[e]Abnormal amniotic fluid includes Polyhydramnios, Oligohydramnios, abnormal smell or colour, etc.
[f]Liver function test includes serum alanine aminotransferase, serum aspartate aminotransferase, albumin, total bilirubin, and conjugated bilirubin.
[g]Renal function test includes serum creatinine and urea nitrogen.

Table 3: The prenatal risk factors of 192903 pregnant women and their fetuses without and with unilateral or bilateral NSCP[a]

Prenatal Risk Factors	No NSCL/P (n=192706)	Unilateral NSCL/P (n=183)	Bilateral NSCL/P (n=14)	P-value
Risk factors exposure in early pregnancy[b]	5733(2.97)	13(7.10)	1(7.14)	0.003
Assisted conception	100(0.05)	1(0.55)	0(0.00)	0.014
Hypertensive disorder	5068(2.63)	6(3.28)	2(14.29)	0.021
Vagina infected[c]	1855(0.96)	6(3.28)	0(0.00)	0.0055
G6PD[d]	996(0.52)	2(1.09)	1(7.14)	0.0014
Incomplete placenta or rough surface placenta	6567(3.41)	40(21.86)	6(42.86)	0
Abnormal amniotic fluid[e]	32258(16.74)	69(37.70)	7(50.00)	0
Low birth weight of newborns	8146(4.23)	71(38.80)	7(50.00)	0
Illness history of mothers				0
No	187173(97.13)	168(91.80)	14(100.00)	
Yes	3022(1.57)	4(2.19)	0(0.00)	
Unknown	2511(1.30)	11(6.01)	0(0.00)	
Prenatal Down's screening				0.025
Low risk	120237(62.39)	94(51.37)	11(78.57)	
High risk	67866(35.22)	83(45.36)	3(21.43)	
Unknown	4603(2.39)	6(3.28)	0(0.00)	
Delivery period of newborns				0
Preterm delivery	9268(4.81)	67(36.61)	7(50.00)	
Term delivery	183002(94.96)	114(62.30)	7(50.00)	
Post-term delivery	436(0.23)	2(1.09)	0(0.00)	

[a]NSCL/P is non-syndromic cleft lip or/and palate.
[b]Risk factors include fever, other sickness, take medicines, radiation exposure, smoke, or drink wine, etc.
[c]Vagina infected fungi, bacteria, trichomonad, or gonorrhea.
[d]G6PD is glucose-6-phosphate dehydrogenase deficiency.
[e]Abnormal amniotic fluid includes Polyhydramnios, Oligohydramnios, abnormal smell or colour, etc.

Table 4: The prenatal high risk factors on NSCL/P[a] were discovered by logistic regression model.

Prenatal Risk Factors	Estimate	Standard Error	Z-value	OR (95% CI)	P-value
Vagina infected[b]	1.23	0.42	2.92	3.43 (1.34, 7.19)	0.003
Assisted conception	2.45	1.02	2.4	11.57 (0.65, 54.29)	0.016
Incomplete placenta or rough surface placenta	1.54	0.18	8.48	4.65 (3.23, 6.57)	0
Abnormal amniotic fluid[c]	1.01	0.15	6.72	2.74 (2.04, 3.67)	0
Folic acid intake <3 bottles (moths) during pre- and early pregnancy[d]	0.45	0.19	2.39	1.57 (1.10, 2.30)	0.017
Low birth weight of newborns	1.69	0.23	7.22	5.44 (3.41, 8.54)	0
Delivery period of newborns					
Term delivery				1	
Preterm delivery	1.19	0.24	5.02	3.29 (2.07, 5.24)	0
Post-term delivery	1.78	0.72	2.48	5.94 (0.97, 18.93)	0.013

[a]NSCL/P is non-syndromic cleft lip or/and palate.
[b]Vagina infected fungi, bacteria, trichomonad, or gonorrhea.
[c]Abnormal amniotic fluid includes polyhydramnios, oligohydramnios, abnormal smell or colour, etc.
[d]There are 31 tablets per bottle, and 0.4 mg folic acid per tablet, the recommended daily intake is one tablet in 3 months before pregnancy and in first 3 months of pregnancy for every pregnant woman.

or post-term delivery. This study strengthens the importance role of interaction between the placental barrier and various environmental factors on the development of NSCL/P.

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Authors's Contributors

BL, XH X, and FH L conceptualised and designed the study, reviewed and revised the manuscript. BL conducted the statistical analysis, drafted the initial manuscript. AC contributed to acquire and sort the data. YL, HF L, and HZ L contributed to sort the data. All authors read and approved the final manuscript.

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Ethics Approval and Consent to Participate

This study was approved by the ethical review board of Guangdong Women and Children Hospital, approval number: 201801023 (decision 2018-07-18). The authors declare that the experiments comply with the relevant guidelines and regulations of ethical review of biomedical research involving human beings issued by Health and Family Planning Commission of the people's Republic of China in 2016. We used the de-identified historical health record data belong to information centre of health bureau of Zhongshan city, and informed consent was waived by the ethical review board of Guangdong Women and Children Hospital.

References

1. Vyas T, Gupta P, Kumar S, Gupta R, Gupta T, et al. (2020) Cleft of lip and palate: A review. J Family Med Prim Care 9: 2621-2625.
2. Sankar SG (2011) Text book of orthodontics. 1st ed. Paras Medical Publication.
3. Allan E, Windson LJ, Stone C (2014) Cleft lip and palate: Etiology, epidemiology, prevention and intervention strategies. Anat Physiol 4: 1-6.
4. Berkowitz S (2006) Cleft lip and palate diagnosis and management. 2nd ed. Springer.
5. Lakhanpal M, Gupta N, Rao NC, Vashisth S (2014) Genetics of Cleft Lip and Palate-Is it still patchy? JSM Dent 2: 1030.
6. Kohli SS, Kohli VS (2012) A comprehensive review of the genetic basis of cleft lip and palate. J Oral Maxillofac Pathol 16: 64-72.
7. Saleem K, Zaib T, Sun W, Fu S (2019) Assessment of candidate genes and genetic heterogeneity in human non syndromic orofacial clefts specifically non syndromic cleft lip with or without palate. Heliyon 5: e03019.
8. Lou S, Ma L, Kan S, Yu X, Wang Y, et al. (2020) Association Study of Genetic Variants in Autophagy Pathway and Risk of Non-syndromic Cleft Lip With or Without Cleft Palate. Front Cell Dev Biol 8: 576.
9. Dixon MJ, Marazita ML, Beaty TH, Murray JC (2011) Cleft lip and palate: understanding genetic and environmental influences. Nat Rev Genet 12: 167-178.
10. Lewis CW, Jacob LS, Lehmann CU (2017) The primary care pediatrician and the care of children with cleft lip and/or cleft palate. Pediatrics 139: e20170628.
11. Dam ET, Der Heijden PV, Korsten-Meijer AGW, Goorhuis-Brouwer SM, Van Der Laan BFAM (2013) Age of diagnosis and evaluation of consequences of submucous cleft palate. Int J Pediatr Otorhinolaryngol 77: 1019-24.
12. Alois CI, Ruotolo (2020) An overview of cleft lip and palate. JAAPA 33: 17-20.
13. Centers for Disease Control and Prevention (2020) Facts about cleft lip and cleft palate.
14. Shkoukani MA, Chen M, Vong A (2013) Cleft lip-a comprehensive review. Front Pediatr 1: 53.
15. Hong Y, Xu X, Lian F, Chen R (2021) Environmental Risk Factors for Nonsyndromic Cleft Lip and/or Cleft Palate in Xinjiang Province, China: A Multiethnic Study. Cleft Palate Craniofac J 58: 489-496.
16. Li H, Luo M, Luo J, Zheng J, Zeng R, et al. (2016) A discriminant analysis prediction model of non-syndromic cleft lip with or without cleft palate based on risk factors. BMC Pregnancy Childbirth 16: 368.
17. Martelli DRB, Coletta RD, Oliveira EA, Swerts MSO, Rodrigues LAM, et al. (2015) Association between maternal smoking, gender, and cleft lip and palate. Braz J Otorhinolaryngol 81: 514-519.
18. Auslander A, McKean-Cowdin R, Brindopke F, Sylvester B, DiBona M (2020) The role of smoke from cooking indoors over an open flame and parental smoking on the risk of cleft lip and palate: A case-control study in 7 low-resource countries. J Glob Health 10: 020410.
19. Kapos FP, White LA, Schmidt KA, Hawes SE, Starr JR (2021) Risk of non-syndromic orofacial clefts by maternal rural-urban residence and race/ethnicity: A population-based case-control study in Washington State 1989-2014. Paediatr Perinat Epidemiol 35: 292-301.
20. Zarante I, López MA, Caro A, García-Reyes JC, Ospina JC (2009) Impact and risk factors of craniofacial malformations in a Colombian population. Int J Pediatr Otorhinolaryngol 73: 1434-1437.
21. Kurita H, Motoki N, Inaba Y, Misawa Y, Ohira S, et al. (2020) Maternal alcohol consumption and risk of offspring with congenital malformation: the Japan Environment and Children's Study. Pediatr Res.
22. Jamilian A, Sarkarat F, Jafari M, Neshandar M, Amini E, et al. Family history and risk factors for cleft lip and palate patients and their associated anomalies. Stomatologija 19: 78-83.
23. Lin Y, Shu S, Tang S (2014) A case-control study of environmental exposures for nonsyndromic cleft of the lip and/or palate in eastern Guangdong, China. Int J Pediatr Otorhinolaryngol 78: 544-550.
24. Chowchuen B, Surakunprapha P, Winaikosol K, Punyavong P, Kiatchoosakun P, et al. (2021) Birth Prevalence and Risk Factors Associated With CL/P in Thailand. Cleft Palate Craniofac J 58: 557-566.
25. Jayarajan R, Natarajan A, Nagamuttu R (2019) Efficacy of Periconceptional High-Dose Folic Acid in Isolated Orofacial Cleft Prevention: A Systematic Review. Indian J Plast Surg 52: 153-159.
26. Millacura N, Pardo R, Cifuentes L, Suazo J (2017) Effects of folic acid fortification on orofacial clefts prevalence: a meta-analysis. Public Health Nutr 20: 2260-2268.
27. Zhou Y, Sinnathamby V, Yu Y, Sikora L, Johnson Cy, et al. (2020) Folate intake, markers of folate status and oral clefts: An updated set of systematic reviews and meta-analyses. Birth Defects Res 112: 1699-1719.

28. Carter TC, Olney RS, Mitchell AA, Romitti PA, Bell EM, et al. (2011) Maternal self-reported genital tract infections during pregnancy and the risk of selected birth defects. Birth Defects Res A Clin Mol Teratol 91: 108-116.

29. Fauque P, De Mouzon J, Devaux A, Epelboin S, Gervoise-Boyer MJ, et al. (2021) Do *in vitro* fertilization, intrauterine insemination or female infertility impact the risk of congenital anomalies in singletons? A longitudinal national French study. Hum Reprod 36: 808-816.

30. Chen L, Yang T, Zheng Z, Yu H, Wang H, et al. (2018) Birth prevalence of congenital malformations in singleton pregnancies resulting from *in vitro* fertilization/intracytoplasmic sperm injection worldwide: a systematic review and meta-analysis. Arch Gynecol Obstet 297: 1115-1130.