



Analysis of Environmental Exposures for Nonsyndromic Cleft Lip and/or Palate: A Case-Control Study

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Abstract

Background: Orofacial cleft is among the most common developmental malformations in humans. This study aimed to identify the relationship between environmental factors and nonsyndromic cleft lip and/or palate (NSCL/P) in Northwest China.

Methods: This case-control study was conducted in Gansu Province, China over two years (Jan. 1, 2017–Jan. 1, 2019). Overall, 600 NSCL/P cases and 660 normal control cases were finally enrolled in the current study. Data were collected by conducting face-to-face interviews with both parents of each case.

Results: Univariate (χ^2) analysis revealed 22 factors as being significantly associated with NSCL/P. Multivariate (stepwise logistic regression) analysis identified that 14 factors had statistically significant association with NSCL/P. Male gender (OR=0.789), paternal age at childbirth of 25–29 yr (OR=0.690), and folic acid supplement (OR=0.197) were found to be protective factors against NSCL/P. On the other hand, blood A-type, multiple births, positive family history of NSCLP (OR=6.660), parental consanguinity (OR=6.107), positive abortion history, high or low maternal childbearing age, and maternal passive smoking (OR=4.349), malnutrition (OR=4.431), infections, and drug use (OR=2.188) during early gestation were significant risk factors for NSCL/P.

Conclusion: Parental age at childbirth of 25–29 yr, and folic acid supplement can reduce the risk of NSCL/P. By contrast, maternal passive smoking, infections, and drug use during early gestation period, and multiple births, parental consanguinity, positive family history, and maternal abortion history can increase the risk of NSCL/P. Identification of risk factors is essential in minimizing the incidence of NSCL/P in a particular population.

Keywords: Nonsyndromic cleft lip; Etiology; Risk factors; Case-control study; Logistic regression analysis



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Introduction

Orofacial cleft is among the most common developmental malformations in humans. It is the most widespread innate abnormality of the head and neck region (1). The demographic and socio-economic status of the population are common factors that affect the incidence of CL/P in certain populations. The etiological risk factors can vary from one country to another or even within the same region (2,3). The worldwide prevalence of CL/P is around 1/700 live births with wide variability among races and regions (2,4). Asian population have more incidence of CL/P than Africans (5).

CL/P can be classified as syndromic and non-syndromic based on their association with specific malformations or their presence as isolated defects, respectively. Both forms of CL/P have a strong genetic component. The syndromic CL/P is mostly the result of chromosomal aberrations or monogenic diseases (6). In contrast, nonsyndromic cleft lip and/or palate (NSCL/P) is a multifactorial disease derived by the interaction between genetic and environmental factors (7). Almost more than half of CL/P cases were found to be nonsyndromic (8). Most of the etiopathogenesis of CL/P has been examined in western societies, but only few cohorts were carried in China and developing countries. China has higher than 20 million childbirths every year. Therefore, based on an investigated prevalence of oral clefts in China (1 in 500 cases), more than 40,000 new subjects of CL/P annually were expected (2,9,10).

Multiple births, blood type, family history, parental childbearing age, and abortion history were recently found to influence the incidence of NSCL/P (2,11). Moreover, maternal exposure to viral infections, drug, tobacco and alcohol use, and radiation exposure during the initial time of gestation were also found to increase the risk of NSCL/P (2,12,13). In contrast, supplementation of folic acid and vitamins in the first trimester of pregnancy were reported to have a reducing role

for NSCL/P (13). While these studies were informative, their applicability are limited to the regions they studied.

Gansu is one of the largest provinces in Northwest China. The incidence of CL/P in Gansu Province has been reported in 1.35/1000 vital births, which is higher than the other provinces of China (14). Accordingly, this study was established in an attempt to assess the potential environmental risk factors associated with NSCL/P in the large Gansu Province population. In addition, this study is the first one in this part of China, with goal that the results can serve as a detailed database for further research and establishment of public health programs concerning prevention of NSCL/P.

Methods

The current study was a case-control study conducted on 600 cleft patients through the Oral and Maxillofacial Surgery department, and 660 normal controls who recruited from Pediatric department of Lanzhou University First Hospital and Gansu Provincial Hospital over two years (Jan. 1, 2017–Jan. 1, 2019).

The ethics representative of the research Institute of Lanzhou University approved this study (No. 201744).

Patients with CL/P and the control subjects free of any congenital anomalies were enrolled in this study. To reduce selection bias, the control group was randomly selected and included more cases than the cleft group. In addition, both patients with CL/P and the control group were matched in their socioeconomic status, region of birth, and ages of less than seven years to reduce recall bias of parents. Exclusion criteria include patients with syndromes or other congenital malformations, unreliable history, and those with adoptive parents.

Through interviews with parents, the children's data were collected, including gender, ABO

blood type, birth order, birth date, and family history of CL/P. Parents' sociodemographic characteristics were also collected, including age, consanguinity, and maternal abortion history. Furthermore, maternal exposure to environmental factors such as malnutrition, tobacco, alcohol, medication use, and folic acid consumption in the first trimester of gestation were also recorded. SPSS ver.25 (IBM Corp., Armonk, NY, USA) was used to accomplish statistical analysis. Univariate analysis was performed using the chi-squared test. Multivariate (stepwise logistic regression) analysis was performed using all the significant factors from the univariate analysis. The results considered significant at $P < 0.05$ and 95% confidence interval level.

Results

Overall, 1260 cases (600 clefts and 660 controls) were included in this study. The mean age was

2.21 (± 2) yr for the cleft group and 2.55 (± 1.9) yr for the control group.

Univariate (χ^2 test) analysis

Compared with control group, males had less risk to be born with NSCL/P ($P=0.045$), while females were at higher risk to have NSCL/P ($P=0.045$). Among ABO blood type, type O was the most common in both the NSCL/P and the control groups, and only blood types A and O were considerably different between the groups. Blood type O was less frequent in NSCL/P than in the control group ($P=0.003$), whereas blood type A was significantly more frequent in NSCL/P group than in the control group ($P < 0.001$). The incidence of increased maternal number of births was more in NSCL/P than the control group ($P < 0.001$). No significant discrepancy regarding the birth season was noticed between the NSCL/P and the control groups. (Table 1).

Table 1: Comparison of child associated factors (χ^2 test)

Variable	Clefts n (%)	Controls n (%)	P value	OR (95% CI)
Sex				
Male	336 (56)	407 (61.7)	0.045*	0.791 (0.632-0.991)
Female	264 (44)	253 (38.3)	0.045*	1.264 (1.009-1.583)
Blood type				
A	171 (28.5)	121 (18.3)	$<0.001^*$	1.776 (1.362-2.315)
B	144 (24)	164 (24.8)	0.743	0.955 (0.738-1.235)
AB	45 (7.5)	55 (8.3)	0.603	0.892 (0.592-1.345)
O	240 (40)	320 (48.5)	0.003*	0.708 (0.566-0.866)
Birth order				
1 st	321 (53.5)	466 (70.6)	$<0.001^*$	0.479 (0.380-0.604)
2 nd and more	279 (46.5)	194 (29.4)	$<0.001^*$	2.088 (1.656-2.633)
Birth season				
Winter	170 (28.3)	187 (28.3)	1.000	1.000 (0.782-1.278)
Spring	169 (28.2)	164 (24.8)	0.201	1.186 (0.923-1.524)
Summer	138 (23)	154 (23.3)	0.894	0.981 (0.755-1.276)
Autumn	123 (20.5)	155 (23.5)	0.221	0.840 (0.643-1.098)
Family history of NSCLP	57 (9.5)	9 (1.4)	$<0.001^*$	7.593 (3.725-15.478)

* show the significant associations

A family history of NSCL/P and maternal aborting history were associated with NSCL/P ($P < 0.001$) (Tables 1 and 2). Parental consanguinity

was significantly associated with NSCL/P ($P < 0.001$) (Table 2). Parental age at childbirth was also significantly correlated with the risk of

bearing a child with NSCL/P. Younger (≤ 19 yr) or elder parents (≥ 35 yr) were at higher risk of giving a birth with NSCL/P. By contrast, parents

aged 25-29 yr were less likely to have a kid with NSCL/P (Table 2).

Table 2: Comparison of parents associated factors at childbearing period (χ^2 test)

Variable	Clefts n (%)	Controls n (%)	P value	OR (95% CI)
Mother age (yr)				
≤ 19	31 (5.2)	14 (2.1)	0.004*	2.514 (1.324-4.773)
20-24	144 (24)	160 (24.2)	0.947	0.987 (0.762-1.278)
25-29	226 (37.7)	311 (47.1)	0.001*	0.678 (0.541-0.849)
30-34	130 (21.7)	145 (22)	0.946	0.982 (0.752-1.284)
≥ 35	69 (11.5)	30 (4.5)	$<0.001^*$	2.729 (1.751-4.254)
Father age				
≤ 19	22 (3.7)	10 (1.5)	0.019*	2.474 (1.162-5.268)
20-24	75 (12.5)	71 (10.8)	0.378	1.185 (0.839-1.674)
25-29	209 (34.8)	307 (46.5)	$<0.001^*$	0.615 (0.490-0.771)
30-34	175 (29.2)	187 (28.3)	0.756	1.042 (0.816-1.330)
≥ 35	119 (19.8)	85 (12.9)	0.001*	1.674 (1.235-2.267)
Consanguinity	20 (3.3)	4 (0.6)	$<0.001^*$	5.655 (1.922-16.641)
Abortion history	140 (23.3)	91 (13.8)	$<0.001^*$	1.903 (1.422-2.546)

* show the significant associations

The maternal common cold or other infections ($P<0.001$), dietary deficiencies ($P<0.001$), and drug use ($P<0.001$) during gestation were associated with NSCL/P (Table 3). Maternal active smoking and drinking did not significantly correlate with a child with NSCL/P in the current project. However, maternal passive smoking was

found to be a significant risk factor for NSCLP incidence ($P<0.001$) (Table 3). Paternal smoking also showed to be a significant risk factor according to our results ($P<0.001$). On the other hand, maternal folic acid supplement was found to be a protective factor against NSCL/P ($P<0.001$) (Table 3).

Table 3: Comparison of maternal conditions and exposures in the 1st trimester of gestation (χ^2 test)

Variable	Clefts n (%)	Controls n (%)	P value	OR (95% CI)
Infections	183 (30.5)	134 (20.3)	$<0.001^*$	1.723 (1.332-2.228)
Fever	42 (7)	45 (6.8)	0.912	1.029 (0.665-1.591)
Anemia	11 (1.8)	6 (0.9)	0.221	2.036 (0.748-5.539)
Diarrhea	16 (2.7)	8 (1.2)	0.065	2.233 (0.949-5.256)
Malnutrition	41 (6.8)	12 (1.8)	$<0.001^*$	3.961 (2.061-7.611)
Drug use	112 (18.7)	59 (8.9)	$<0.001^*$	2.338 (1.669-3.275)
Folic use	227 (37.8)	480 (72.7)	$<0.001^*$	0.228 (0.180-0.289)
Maternal				
Active smoking	8 (1.3)	5 (0.8)	0.406	1.770 (0.576-5.441)
Drinking	8 (1.3)	12 (1.8)	0.510	0.730 (0.296-1.798)
Passive smoking	223 (53.8)	140 (21.2)	$<0.001^*$	2.167 (1.712-2.820)
Paternal				
Smoking	320 (53.3)	247 (37.4)	$<0.001^*$	1.911 (1.526-2.393)
Drinking	300 (50)	310 (47)	0.284	1.129 (0.905-1.409)

* show the significant associations

Multivariate (multiple stepwise logistic regression) analysis

The 22 significant factors identified in the univariate analysis were enrolled in multiple stepwise logistic regression. Among 22 factors, 14 were significant in the final model, entered into the regression equation. The regression coefficients and ORs (with 95% CI) were estimated for these factors (Table 4). The findings confirmed that the male gender (OR=0.789, 95% CI=0.624-0.996), paternal childbearing age of 25–29 yr, and use of folic acid supplementation (OR=0.197, 95% CI: 0.151–0.256) were found to be significant factors for reducing the incidence of a child with NSCL/P. In contrast, blood type A (OR=1.205, 95% CI: 1.100–1.320), second birth or more

(OR=1.987, 95% CI: 1.566–2.520), positive family history (OR=6.660, 95% CI: 3.244–13.673), previous abortion (OR = 1.974, 95% CI: 1.467–2.655), high or low maternal age at childbearing (OR_{high}=2.360, 95% CI: 1.488–3.740; OR_{low}=2.701, 95% CI: 1.410–5.175), parental consanguinity (OR=6.107, 95% CI: 2.048–18.210), maternal exposure to infections, malnutrition, and passive smoking during pregnancy (OR=1.480, 95% CI: 1.037–2.114; OR=4.431, 95% CI: 2.163–9.079; OR=4.349, 95% CI: 3.319–5.699, respectively), and drug use during gestation (OR=2.188, 95% CI: 1.401–3.416) were found to be significant risk factors for the incidence of a child developing NSCL/P.

Table 4: Results of multiple stepwise logistic regression analysis

Variable	B	P value	OR (95% CI)
Male	-0.237	0.047*	0.789 (0.624-0.996)
Blood type A	0.187	<0.001*	1.205 (1.100-1.320)
≥2 nd birth	0.687	<0.001*	1.987 (1.566-2.520)
Family history	1.896	<0.001*	6.660 (3.244-13.673)
Mother age(yr)			
≤19	0.994	0.003*	2.701 (1.410-5.175)
≥35	0.858	<0.001*	2.360 (1.488-3.740)
Father age			
25-29	-0.372	0.002*	0.690 (0.544-0.875)
Consanguinity	1.809	0.001*	6.107 (2.048-18.210)
Aborting history	0.680	<0.001*	1.974 (1.467-2.655)
Infections	0.392	0.031*	1.480 (1.037-2.114)
Malnutrition	1.489	<0.001*	4.431 (2.163-9.079)
Drug use	0.783	0.001*	2.188 (1.401-3.416)
Folic use	-1.627	<0.001*	0.197 (0.151-0.256)
Passive smoking	1.470	<0.001*	4.349 (3.319-5.699)

B; Regression coefficient - * show the significant associations

Discussion

The current study is the first study to evaluate the risk factors associated with NSCL/P in Northwest China; Gansu Province. Our research was conducted in the First Hospital of Lanzhou University and Gansu Provincial Hospital, which are the referral hospitals for the whole Province with approximately 27 million people.

The relationship between gender and the incidence of NSCL/P was reported in few studies. However, such relationship might be a direct reflection of the gender distribution in the surrounding population. Similar to Lin et al. (11), we found that men had less risk than women to be born with NSCL/P compared with the control group. Although the studies assessing the relation of NSCL/P with blood type are rare, the existing

studies found a significant relationship between blood type and NSCL/P. The present study found that blood type O was more frequent in the control group than NSCL/P, while blood type A had more frequency in NSCL/P than the control group, which was consistent with the findings of Meng et al. (2), but different than another study (11). The mechanism of how blood species could affect union of the upper lip and palate require further research for understanding such relationship.

In the present study, increasing births number was correlated with a higher risk of NSCL/P, and this also was reported by other studies. For instance, a relatively significant rate of NSCL/P occurred was found in spouses having three or more previous childbirths (15). Similarly, a large proportion of NSCL/P cases were the births after two or more previous births, which was statistically significant (16). This relationship may be justified as the multiple births may result in alteration of reproductive organs.

Our results demonstrated no significant correlation between seasonal birth and NSCL/P, confirmed by Siffel et al. (17) and Lin et al. (11). However, among the NSCL/P group and regardless of gender differences, the incidence of NSCL/P was found to be higher in Winter and Spring, which was similar with the other results (12, 18). In contrast, the higher proportion of NSCL/P was recorded through the spring and summer (19). These results may be attributed to variable study designs, cultural and lifestyle differences in geographically distinct populations.

The current cohort was similar to many reports, revealed significant implication of positive family history and parental consanguinity in increasing the risk of NSCL/P (4,11). A positive family history of CL/P was significantly associated with incidences of births with cleft lip with or without palate (CL-P) and cleft palate only (CPO). In addition, parental consanguinity was also correlated with bearing children with CL-P. Another study also found the positive family history to be correlated more with children with CL-P than with CPO (20). Furthermore, it was mainly positive in children of consanguineous parents compared to

those of nonblood relation parents. Most of the genetic clefts have occurred when consanguinity and family history simultaneously found in a family. The current study was comparable with others, in which the mothers who had a history of abortion were at high risk of having a baby with NSCL/P (11). The impaction of previous aborting in CL/P could be explained by the firm relation between the abortion and the TT genotype of rs2235373 in interferon regulatory factor 6 (IRF 6) (21).

The parental age at childbirth in this study had implications on NSCL/P incidence, which was in agreement with other studies (12,22). Our results revealed that parents with high or low ages (≥ 35 , ≤ 19) were at higher risk of having a birth with NSCL/P. Similarly, the findings of a meta-analysis indicated that fathers aged 40 yr or more and mothers aged 35 yr or higher had a high risk of having CL/P offspring than those within 20 to 29 yr old (23). Another study, higher incidence of NSCL/P was in younger mothers and elder parents, while the middle-aged parents had the lowest incidence (25–29 yr) (11). Similar results reported in that elder fathers were at higher risk of having a child with NSCL/P; however, such relationship was not found in different maternal ages in their study (24).

Development and union of upper lip and palate mostly happens in the initial time of gestation. This period is regarded as a delicate phase of development. Our study was in line with others, in which infections during the early gestation was a significant risk factor for NSCL/P (2). In addition, we found the deficiency of nutrients during this period to be associated with the risk of NSCL/P occurrence. This finding was supported by many other studies (16). Some cohorts also had noted that babies with CL/P have significantly less weight compared with healthy infants of the same growing generation. The present study found that maternal drugs use had potential implications in the impaction of embryo growth, and inhibition of the lip and palate merging resulting in a CL/P, which was similar to the results of other studies (10,11). While the exact mechanism was still unclear, the findings of some

cohorts indicated that use of medication was observed to combine with destructive genes that result in NSCL/P. For instance, phenytoin combines *Satb2* and *Hoxa2* (25), and dexamethasone interacts with the *Fgf10* signal pathway (26), which may increase the risk of NSCL/P development.

The use of tobacco and alcohol in the prenatal period were recognized as factors linked with NSCL/P births (16). Our findings regarding the relationship between smoking and alcohol, and risk of NSCL/P occurrence however demonstrated that neither maternal smoking, drinking, nor paternal alcohol use had a significant association with NSCL/P. The same results were reported by many other researchers (11, 27). Although China has one of highest number of nicotine users, including more than 300 million smokers, only 3.8% of smokers are females. In our study, paternal smokers were noted to be at higher risk of having a child with NSCL/P, and this result was supported by other cohorts (28).

Passive smoking was also found to be a significant risk factor for NSCL/P occurrence, supported by other cohorts (29). Passive smoking may raise the chance of NSCL/P more than active smoking (30). A probable explanation for this finding may be because a smoking woman often will cease tobacco during gestation, but may not be aware of the danger of passive smoking on embryo development. Wang et al. (10) reported limited prenatal smoking among the Chinese people (2%), but a high rate of women (28.7%) are exposed to passive smoke, correlated with a higher risk of NSCL/P development.

The importance of folic acid in prenatal care has been identified as protective factor against the risk of NSCL/P development. This was supported both in our cohort and other studies (13). Therefore, folic acid supplementation, including dose intake no less than 400 mg/day is a valuable approach that could significantly decrease the incidence of NSCL/P. This may be one of the modifiable factors with efficient public instruction plans to reduce the prevalence of orofacial cleft on a population scale.

Conclusion

Supplementation of folic acid, adequate nutrition during pregnancy, and suitable age of couples during childbearing (25-29 y) are associated with a reduced risk of NSCL/P. By contrast, maternal drug use and exposures to infection, malnutrition, and passive smoking during the initial time of gestation were risk factors associated with NSCL/P. Additionally, positive family history of CL/P, maternal abortion history, multiple births, high or low parental childbearing age, and parental consanguinity were identified as risk factors in the development of NSCL/P. A thorough understanding for the association between the risk factors and NSCL/P stills unclear and additional researches need to be conducted to unmask the etiology of NSCL/P.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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