Letter to the Editor

A Case-control Study of Environmental Risk Factors for Nonsyndromic Cleft of the Lip and/or Palate in Xuzhou, China*

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In this study, we sought to determine the association between environmental factors and nonsyndromic cleft of the lip and/or palate (NSCLP) to understand the etiology of the disease. A total of 200 NSCLP cases and 327 controls were recruited at the Maternal and Child Health Hospital of Xuzhou City. We conducted face-to-face interviews with the mothers of both cases and controls. The factors increasing the risk of NSCLP were a positive family history [odds ratio (OR)=56.74], pesticide exposure (OR=8.90), and indoor decoration pollution (OR= 4.32). On the other hand, the factors decreasing the risk of NSCLP were a high education level (OR=0.22) and supplementation of folic acid (OR=0.23) and multivitamins (OR=0.16). Positive family history, pesticide exposure, and indoor decoration pollution are associated with the risk of NSCLP. In contrast, high education level and folic acid and multivitamin supplementation are protective factors against NSCLP.

Clefts of the lip and/or palate (CLPs) are among the most common birth defects worldwide. The global incidence of CLP is approximately 1 in 700[1]. Persons with CLP may experience problems with feeding, speaking, hearing, and social integration, which may reduce their quality of life. CLP may also result in increased mortality and morbidity. CLP is becoming an important public health and social problem.

CLP is divided into cleft lip with or without cleft palate (CL/P) and cleft palate only (CPO). Approximately 70% of CL/P cases and 50% of CPO cases occur as isolated defects with no other apparent cognitive or structural abnormalities, and are termed nonsyndromic CLP (NSCLP). Because the etiology of NSCLP is complicated with both genetic and environmental contributions, it is difficult to identify specific etiologic factors. Several potential risk factors have been studied; however, no strong risk factor has yet been identified[2].

Chinese newborns present a relatively high birth prevalence of NSCLP[3]. Because higher rates of NSCLP were observed in China, more studies need to be conducted. However, most epidemiological studies on NSCLP derive from studies carried out in Western countries. Recently, a case-control study was conducted in eastern Guangdong[4], a province in southern China. Because populations in different areas may be exposed to different environmental factors, environmental factors that increase the risk of NSCLP in other regions must be identified.

Xuzhou is a city in Jiangsu Province, China, located in the southeast of the North China Plain. NSCLP is also a great public health concern in this region. The environment, biology, personal behavior, and lifestyle pattern in the region are different from those in eastern Guangdong. Therefore, the environmental risk factors for NSCLP may also be different between the two regions. It is important to disclose the etiology of this disease in this region, and to design protocols based on dealing with environmental factors.

To identify the environmental risk factors for NSCLP, we carried out a case-control study to determine the relation between environmental factors and NSCLP. The results of this study will help in better understanding NSCLP and in laying a solid foundation for the prevention of NSCLP in Xuzhou.

The hospital-based case-control study was performed at the Maternal and Child Health Hospital

doi: 10.3967/bes2015.076

*This study was supported by the National Natural Science Foundations of China (No. 81273103) and the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

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of Xuzhou City, China, from July 2012 to July 2014. The study was approved by the ethical committee for Human Research of Xuzhou Medical College. The NSCLP cases were diagnosed by using prenatal B-ultrasonography. Subjects exhibiting syndromes or other congenital malformations were excluded. The control subjects were infants born in the same hospital during the same period without any birth defects or systemic disease. The sample size was calculated by using the following formula: $n=(1+1/c)\,\overline{pq}\,(Z_{\alpha}+Z_{\beta})^2/(p_1-p_0)^2$. The signed informed consent sheets were obtained from the parents of the subjects. We conducted face-to-face interviews with the mothers of both cases and controls at the hospital by using a questionnaire.

Statistical analyses were performed by using the Statistical Product and Service Solutions 18.0 (SPSS Inc., Chicago, IL, USA). The enumeration data were evaluated by using χ^2 tests. Multiple logistic regression analyses were used for the multivariate determination of parameters affecting NSCLP. The variable were divided into three groups or more to deal with dummy variable. Odds ratios (OR) were reported along with 95% confidence intervals (CI). Two sided *P*-values <0.05 and the OR with 95% CI that excluded CI =1 were judged to be statistically significant.

According to the formula, the sample size of the case group was 141 and that of the control group was 230. To obtain more valid data, a total of 527 subjects including 200 cases and 327 controls were recruited. Cases of cleft lip with cleft palate (CL+P) were the most common (*n*=142, 71%), followed by cleft lip without cleft palate (CL, *n*=40, 20%) and CPO (*n*=18, 9%).

The mean maternal childbearing age in the case and control groups was 26.87±6.25 and 26.49±5.53 years, respectively. Childbearing age was significantly associated with an increased risk of NSCLP. Maternal education may influence the risk of NSCLP. There was a significantly increased risk of having a child with NSCLP in mothers with a low level of education than in those with high levels of education. Mothers with normal blood pressure had a lower risk of NSCLP than mothers with a high blood pressure or low blood pressure level. Maternal history of abortion was significantly associated with NSCLP. A positive family history was a significant risk factor for NSCLP (Table 1).

Maternal drinking in early pregnancy was significantly associated with an increased risk of NSCLP (OR=9.39). This study showed that 8 (4.6%) of the case mothers had smoked in the first 3 months of pregnancy, whereas only 1 (0.3%) control mother had smoked. There was a significant association between maternal tobacco use and NSCLP (OR=15.36). More than half of the case mothers (56.9%) had passive smoking during early pregnancy, which is higher than that in the control mothers (χ^2=55.42, *P*<0.05). Passive smoking was associated

Table 1. Association between Maternal Characteristics and NSCLP at the Time of Childbirth in Xuzhou from 2012 to 2014

Characteristics	Case *n* (%)	Control *n* (%)	χ^2	*P*
Childbearing age (y)				
≤19	9 (4.5)	14 (4.3)		
20-	76 (38.4)	71 (21.8)	17.29	0.001
25-	67 (33.5)	148 (45.3)		
≥30	46 (28.3)	92 (28.3)		
Education				
Senior high school and above	71 (36.4)	241 (73.9)	71.49	<0.001
Junior high school and below	124 (63.6)	85 (26.1)		
Blood pressure				
Low	13 (4.5)	13 (6.0)		
Normal	178 (89.0)	313 (95.7)	13.70	0.001
High	9 (4.5)	1 (0.3)		
Abortion history	84 (42.2)	133 (40.7)	8.18	0.04
Family history	25 (14.9)	3 (1.5)	23.94	<0.001

with an increased risk of NSCLP (OR=4.05). The percentage of exposure to indoor decoration pollution in case mothers was higher than that in control mothers (χ^2=16.66, P<0.05). A statistically significant association was found between maternal indoor decoration pollution and NSCLP (OR=2.95). There was a significantly increased risk of having a child with NSCLP in mothers with contraceptive use compared with mothers without contraceptive use (OR=2.09) (Table 2).

In the study, 82.6% of mothers in the control group had folic acid supplementation in the first trimester of pregnancy, whereas only 45.4% of case mothers had folic acid supplementation (χ^2=74.2, P<0.05). Folic acid supplementation was found to exhibit a protective influence against NSCLP (OR=0.18). Supplementation of calcium was not significantly associated with NSCLP (OR=0.92). A significant association was found between maternal supplementation of vitamin A and NSCLP (OR=16.46). Taking multivitamins during pregnancy was a protective factor against NSCLP (OR=0.18) (Table 2).

We chose 21 significant variables that were identified with χ^2 (P<0.05) analysis to enter into multiple logistic regression. The variable were divided into three groups or more to deal with dummy variable. The final model identified six significant variables that were entered into the regression equation (Table 3). The multiple logistic regression confirmed that the factors increasing the risk of NSCLP were a positive family history (OR=56.74), pesticide exposure (OR=8.90), and indoor decoration pollution (OR=4.32). 'Family history' refers to having at least one relative with NSCLP in five generations of family members. 'Pesticide exposure' refers to mothers who were exposed to pesticides one or more times during the first 3 months of pregnancy. 'Indoor decoration pollution' refers to mothers who lived in a house decorated or added with new furniture for no more than half a year covering the first 3 months of pregnancy. On the other hand, the factors decreasing the risk of NSCLP were high education level (OR=0.22) and supplementation of folic acid (OR=0.23) and multivitamins (OR=0.169).

Our study indicated that a positive family history significantly influenced the risk of having a child with

Table 2. Association between Environmental Factors and NSCLP in Xuzhou from 2012 to 2014

Factors	Case *n* (%)	Control *n* (%)	χ^2	*P*	OR	95% CI
Drink	16 (8.0)	3 (0.9)	17.91	<0.001	9.39	2.70-32.66
Smoking	9 (4.5)	1 (0.3)	11.73	0.001	15.36	1.93-122.19
Passive smoking	111 (55.5)	77 (23.5)	55.21	<0.001	4.05	2.77-5.91
Pesticide exposure	46 (23.0)	12 (3.7)	47.34	<0.001	7.84	4.04-15.23
Indoor decoration pollution	51 (25.5)	34 (10.4)	20.92	<0.001	2.95	1.83-4.75
Contraceptive use	44 (26.0)	47 (14.4)	10.12	0.001	2.10	1.32-3.33
Folic acid	91 (45.5)	270 (82.6)	79.03	<0.001	0.18	0.19-0.26
Calcium	84 (42.0)	144 (44.0)	0.21	0.647	0.92	0.65-1.31
Vitamin A	18 (9.0)	2 (0.6)	24.06	<0.001	16.46	3.74-72.45
Multivitamins	13 (6.5)	91 (27.8)	35.64	<0.001	0.18	0.10-0.33

Table 3. Results of Multiple Logistic Regression Analysis of Environmental Factors for NSCLP in Xuzhou from 2012 to 2014

Factors	B	SE	Wald	df	Sig.	OR	95% CI
Family history	4.04	1.23	10.84	1	0.001	56.74	5.13-628.19
Pesticide	2.19	0.81	7.26	1	0.006	8.90	1.81-43.59
Indoor decoration pollution	1.47	0.60	5.90	1	0.015	4.32	1.33-14.07
Education	-1.50	0.43	12.02	1	0.001	0.22	0.10-0.52
Folic acid	-1.47	0.44	11.10	1	0.001	0.23	0.10-0.55
Multivitamins	-1.85	0.67	7.58	1	0.006	0.16	0.04-0.59

NSCLP, which is similar to the results of other previous studies[5]. As a genetic disease, subjects with NSCLP are more prone to be born in families with a positive history. A positive family history of NSCLP implies the very strong role of genetic factors in NSCLP. More considerable interest needs to be developed to identify the genes contributing to the etiology of NSCLP.

In this study, we found a significant association between pesticide exposure and NSCLP, in line with other studies. A recent study showed that the herbicide trifluralin was associated with CLP risk[6]. To better understand the relation between pesticide exposure and NSCLP, evaluating multiple routes of maternal exposure and individual genetic susceptibility should be considered in future studies.

The study indicated that maternal exposure to indoor decoration may confer an increased risk of giving birth to an infant with NSCLP. No data about the association between indoor decoration pollution and NSCLP have been reported previously. Housing renovation has become a new source of indoor environmental pollution, which may be a threat to health, especially for fetuses and children. A study has reported the association between indoor renovation and congenital heart disease[7]. It is necessary to gain insight into the specific effects of indoor decoration pollution on the risk for NSCLP.

Our study showed that a high education level was a protective factor against NSCLP. The data indicated that parents with a low education level tended to smoke more and had less healthy diets than those with higher education levels. Therefore, a low education level seemed to be a risk factor for NSCLP[4]. This study confirmed the relation between maternal education and NSCLP.

The potential preventive effects of folic acid supplementation on NSCLP are similar to those in other studies[8]. Li et al. showed a reduced risk for CL/P among women who had used periconceptional folic acid in a northern rural region of China[8]. Our study also found a reduction of the risk of NSCLP with multivitamin supplementation. In a meta-analysis, multivitamin use reduced the birth prevalence of CLP by 25%[9]. Therefore, use of multivitamin supplements in early pregnancy has been linked to a decreased risk of CLP. However, multivitamins in the absence of folic acid showed a weakly protective effect[10]. Therefore, it is suggested that the preventive effects of multivitamins on NSCLP may benefit from folic acid supplementation.

The study was aimed at examining the environmental risk factors for NSCLP in Xuzhou, China. Positive family history, pesticide exposure, and indoor decoration pollution are among the risk factors associated with NSCLP. High education level and supplementation of folic acid and multivitamins play a beneficial role in reducing the prevalence of NSCLP. This research will help set up targeted measures based on dealing with environmental factors.

We wish to thank the Maternal and Child Health Hospital of Xuzhou City for recruiting patients.

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Received: February 3, 2015;
Accepted: May 4, 2015

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