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Maternal environmental factors and nonsyndromic cleft lip and palate in a Brazilian population

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Abstract

Aim: Cleft lip and/or palate (CL/P) represent the most common congenital anomalies of the face and shows a multifactor etiology. The purpose of this study was to evaluate the relationship between maternal environmental factors and CL/P.

Methods: The study design was an epidemiological case-control single-center study. All participants were recruited from the same institution. We interviewed 1,212 mothers, who were divided into 2 groups: (1) *cases*: mothers of children with CL/P (n=606), and (2) *controls*: mothers of children without CL/P (n=606). The information collected was stored in a database and analysed using statistical program SPSS® version 19.0. Statistical analysis was initially based on the presentation of descriptive data. To determine association among maternal environmental factors, and occurrence of CL/P, prevalence odds ratios were calculated.

Results: Of the 606 patients with clefts, 340 (56.10%) were born with CLP, 157 (25.9%) with CL and 109 (17.98%) with isolated CP. Of all participants (n=1,212), 225 (18.6%) presented a positive history of cleft in their families and 975 (80.4%) presented a negative history and 12 (1%) not inform. Maternal smoking and alcoholism in the first trimester of pregnancy were significantly associated with the occurrence of CL/P (p= 0.000 and p=0.001, respectively). With respect to maternal use of medicines during the first trimester of pregnancy, use of amoxicillin showed an increased risk of 2.04 times higher for development of CL/P (95% CI: 1.06-3.93, p=0.02). There was no significant association between other medicines and illicit drugs in the first trimester of pregnancy and the occurrence of clefts in this population.

Conclusion: Our results found association between maternal smoking, alcoholism and use of amoxicillin in the first trimester of pregnancy and CL/P. The identification of modifiable risk factors for CL/P is the first step toward primary prevention.

Introduction

The embryological development of the face relies on the interplay of a vast range of factors encompassing cell differentiation, growth, apoptosis, cell to cell adhesion, and inter and intracellular signaling. The disruption of a gene that control one or more of these factors, inhibition of cell function by environmental teratogens or a combination of the two is likely to be the etiological cause of craniofacial malformations such as nonsyndromic cleft lip with or without cleft palate (NSCL/P) [1].

NSCL/P (OMIM #119530) represents the most frequent congenital malformation in the head and neck region, with a prevalence ranging from 1:500 to 1:2500 live births [2]. Its prevalence varies according to ethnicity (Africans: 0.3:1,000; Europeans: 1.3:1,000; Asians: 2.1:1,000; Native Americans: 3.6:1,000) and socioeconomic level [2]. In Brazil, the prevalence of NSCL/P ranges from 0.36 to 1.54:1,000 live births [3,4].

Approximately 70% of CL with or without CP and 50% of CP is considered nonsyndromic, *i.e.*, consisting of isolated anomalies with no other apparent cognitive or structural abnormalities [5]. Although not a major cause of mortality in developed countries, NSCL/P does cause considerable morbidity in affected children and imposes a substantial financial burden especially for families of low socioeconomic status [6]. Individuals with NSCL/P are subject to difficulties with eating, speaking, hearing, social integration and cancer [5,7].

NSCL/P are caused by a complex interplay between environmental exposures, genetic and epigenetic factors. Although in the past decade multiple genetic variants were associated with NSCL/P, providing valuable insights into its genetic etiology, the disease-susceptibility

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genes identified so far only account for a small percentage of cases [8]. The identification of modifiable risk factors for NSCL/P is the first step toward primary prevention. Risk factors such as maternal exposure to tobacco smoke, alcohol, poor nutrition, gender, maternal age, and medicinal drugs in the workplace or at home in early pregnancy have previously been investigated [8-11].

Antibiotics are frequently prescribed during pregnancy [12]. In observational studies, maternal use of antibiotics has been associated with an increased risk of different birth defects, including NSCL/P [13,14]. Among antibiotics, amoxicillin has been the preferred drug for the treatment of respiratory and urinary tract infections [15], conditions frequently encountered in women of childbearing age. Thus, it is particularly important for clinicians, pregnant women and public-health officials to have information on the potential teratogenic effects of amoxicillin in order to make risk-benefit judgments on its use early in pregnancy [16].

Since environmental factors play a role of major importance in the etiology of oral clefts, the purpose of this study was to investigate its relation with the occurrence of NSCL/P in a Brazilian subpopulation.

Methods

The study design was an epidemiological case-control single-center study. All participants were recruited from the same institution (Centre for Rehabilitation of Craniofacial Anomalies, Minas Gerais State, Brazil), between August 2013 and September 2015, in an attempt to select cases and control individuals with similar ethnicities and social-culture backgrounds. The aforementioned Reference Center treats exclusively patients from the public health care system (SUS), being certified by the Ministry of Health.

We interviewed 1,212 mothers, who were divided into 2 groups: (1) *cases*: mothers of children with NSCL/P (n=606), and (2) *controls*: mothers of children without NSCL/P (n=606) (these patients were assisted in General Clinics). All mothers were further classified for the presence or not presence of environment factors during the first trimester of pregnancy.

All of the subjects were from Minas Gerais, Brazil, where there is an admixed population of Europeans (mostly from Portugal and Italy) and Africans, with a small percentage of native Brazilian Indians [17]. With regard to skin color, most of the patients in both groups were non-Caucasian.

All cleft patients were diagnosed independently and screened for the presence of associated anomalies or syndromes by a multidisciplinary team of specialists. From both groups we excluded the mothers who did not accept to participate in the study, children with syndromes and parents from consanguineous marriages. Mothers who were unsure of contact with environment factors in the first trimester of pregnancy were also excluded.

The interviews were conducted at a single time and after the scheduled attendance at that session. The interview includes questions on family history of clefts, maternal exposure to tobacco smoke, alcohol and illicit drugs, and maternal use of medicines, including anti-inflammatory, antibiotics or anticonvulsants. Mothers with exposure to tobacco and alcohol were classified as users or non-users. To the question on use of medicines was presented a list with the trade names available. We defined exposure as systemic use of medicines during the first trimester of pregnancy. The measurements were undertaken by a team of trained research assistants at Center for Rehabilitation of Craniofacial Anomalies. Intraclass correlation coefficients were calculated to check concordance intra and interobserver. The concordances were high and significant.

The clefts were categorized into three groups with the incisive foramen as reference: (1) Cleft Lip (CL): includes complete or incomplete pre-foramen clefts, either unilateral or bilateral; (2) Cleft Lip and Palate (CLP): includes unilateral or bilateral transforamen clefts and pre- or post-foramen clefts; (3) Cleft Palate (CP): includes all post-foramen clefts, complete or incomplete [18].

The information collected was stored in a database and analysed using statistical program SPSS® version 19.0 (*Statistical Package for Social Sciences for Windows, Inc., USA*). Statistical analysis was initially based on the presentation of descriptive data. To determine association among maternal environment factors, and occurrence of NSCL/P, prevalence odds ratios were calculated. Analyses were then performed, using the chi square test, with a confidence interval of 95% and p value ≤0.05 considered statistically significant. Then also was tested the association between the family history of clefts and the occurrence of NSCL/P.

This study was approved by the Ethics Committee in Research of the University (761.621/2014). All patients were informed about the study's purpose before they consented to participate.

Results

A total of 1,212 women were interviewed including 606 mothers of children with NSCL/P (cases) and 606 mothers of infants with no major birth defects (controls). Of 1,212 children included in the analysis, there was a predominance of males (54.95%). In the group case, 274 (50.18%) were female and 332 (49.84%) male, while, in the control group, 272 (49.82%) children were female and 334 (50.16%) male. No differences were found between groups (p=0.908) (Table 1).

Of the 606 patients with clefts, 340 (56.10%) were born with

Table 1. Factors associated with the occurrence of nonsyndromic cleft lip and/or palate between the case and control groups.

Variables	Case n (%)	Control n (%)	OR	IC	*p*-value
Gender					
Female	274 (50.18)	272 (49,82)			
Male	332 (49.84)	334 (50.16)	1.01	0.80-1.27	0.908
Family history of cleft					
Yes	184 (30.9)	41 (6.80)			
No	411 (69.1)	564 (93.2)	6.16	4.29-8.84	0.000
Maternal exposure					
Cigarette smoking					
Yes	97 (16.4)	33 (5.50)			
No	494 (83.6)	568 (94.5)	3.38	2.23-5.10	0.000
Alcohol consumption					
Yes	59 (14.18)	45 (7.75)			
No	357 (85.82)	535 (92.25)	1.96	1.30-2.96	0.001
Illicit drugs					
Yes	7 (1.68)	3 (0.52)			
No	408 (98.32)	577 (99.48)	3.30	0.84-12.84	0.06
Drug consumption*					
Yes	45 (7.42)	53 (8.74)			
No	561 (92.58)	553 (91.26)	0.83	0.55-1.26	0.39

***Drug consumption:** defined as mother that took medicines, such as anti-inflammatory, antibiotics or anticonvulsants (barbiturates or benzodiazepines), in the first trimester of gestation

CLP, 157 (25.9%) with CL and 109 (17.98%) with isolated CP. Of all participants (n=1,212), 225 (18.6%) presented a positive history of cleft in their families and 975 (80.4%) presented a negative history and 12 (1%) not inform. Of 225 patients with positive family history of clefts, 184 (81.8%) were in the case group and 41 (18.2%) in the control group (p=0.000).

Of 1,212 mothers, 1,192 (98.4%) was assessed for tobacco use in the first trimester of pregnancy, these, 591 (49.6%) were cases and 601 (50.4%) were controls. The most evaluated mothers (1.062; 89.1%) have not had exposure to tobacco. Into the case group, a greater proportion of mothers (97; 16.4%) were exposed to tobacco in the first trimester of pregnancy compared to control group, where only 33 (5.5%) them were exposed to tobacco in the first trimester pregnancy (p=0.000). Considering the association between maternal smoking and clefts in gender separately, also, was founded association in both genders, female (p=0.004) and male (p=0.000).

Of 1,212 mothers, 996 (82.2%) was assessed for alcohol use in the first trimester of pregnancy, 416 (41.8%) cases and 580 (58.2%) controls. There was association between cases and controls (p=0,000), with greater proportion of cases (59 – 14.18%) than controls (45 – 7.75%) with alcohol use during the first trimester of pregnancy. Evaluating the genders separately, the association between maternal alcohol using and clefts was significant in both, females (p=0.037) and males (p=0.009).

Concerning the use of medication during the first trimester of pregnancy, 83 (84.69%) administered antibiotics; 13 mothers (13.26%) reported having used anti-inflammatory; 2 (2.05%) used anticonvulsants. No significant difference was observed in the case and control groups (Table 2).

With respect to maternal use of amoxicillin, of 1,212 mothers, 1,165 (96.1%) was assessed, these, 573 (49.2%) cases and 592 (50.8%) controls. The proportion the mothers with amoxicillin use in the first trimester of pregnancy was greater in the cases (27; 4.72%) than in the controls (14; 2.37%) (p=0.02). The results are presented in Table 1.

Discussion

Most nonsyndromic oral clefts are thought to be caused by interaction between polygenic and environmental factors, but the exact mechanisms remain unknown [8,19]. The patients described in this study were recruited from the Centre for Rehabilitation of Craniofacial Anomalies, Minas Gerais State, Brazil. This Service is considered one of the largest cleft repair centres in Brazil, and performs all procedures of rehabilitation that pass through the Brazilian Public Health System [9,10]. With a population exceeding 190 million people and 3 million babies born every year, NSCL/P is an important public health problem in Brazil, with approximately 4,000 new cases of clefts every year [10].

In the present study, the group with NSCL/P included 332 (54.78%) male and 274 (45.22%) female. Of the 606 patients with clefts, 340 (56.10%) were born with CLP, 157 (25.9%) with CL and 109 (17.98%) with isolated CP. In a previous study that we carried out with the Brazilian population, 126 patients with oral cleft showed a 1.3 ratio of males to females. Males were 2.57 times more affected by CLP than females. CLP, with a prevalence of 39.68%, and isolated CL, with a prevalence of 38.09%, were the most common anomalies, followed by isolated CP (22.23%) [3]. In another recent study by us also in a Brazilian population, involving 843 patients with oral clefts, 474 (56.2%) were male and 369 (43.8%) were female [11]. With respect to the distribution of clefts, it was found that of the 843 NSCL/P, 680 (80.7%) were CL/P and 163 (19.3%) were isolated CP [11]. These results confirm the findings of this study.

With respect to family history for oral clefts, our study showed that 225 (18.60%) patients presented a positive history of cleft in their families. Of 225 patients with positive family history, 184 (81.80%) were in the case group and 41 (18.20%) of the control group. In a study in Brazil, between 2004-2008 years, a positive familial history of cleft malformation was found in 35.13% of cases [20]. A study investigating 4,557 affected children born in Czechoslovakia over a period of 29 years, registered a positive familial history in 18% of cases [21]. In one study comprised of 540 individuals with NSCL/P in Poland, also registered a positive familial history in 18% of cases [22]. In another study comprised of 153 individuals with NSCL/P in Thailand, registered a positive familial history in 17.7% of cases [23]. The results of these various studies in different countries, showed results similar to our observed in a Brazilian population.

Maternal smoking has long been considered a risk factor for the occurrence of oral clefts [8,11,24]. In our study, the mothers group case (n=591), 97 (16.40%) were exposed to smoking in the first trimester of pregnancy, while in the control group, of 601 mothers, only 33 (5.50%) were exposed to smoking in the first trimester pregnancy (p=0.000). This result is relevant and consistent with previous literature data. In a previous study, we evaluated 1,519 mothers, and 25% of them in the case group were smoker. There was an association between maternal smoking and clefts (odds ratio, OR=2.02, CI95%, 1.54–2.63). Maternal smoking was also associated with CLP (OR= 2.08, CI95%, 1.58–2.75) and with CP (OR=1.92, CI95%, 1.26–2.92)11. Honei *et al* (2007) [24] have shown that periconceptional smoking was associated with CLP

Table 2. Medicines associated with the occurrence of nonsyndromic cleft lip and/or palate between the case and control groups.

Variables	Case n (%)	Control n (%)	OR	IC	*p*-value
Anti-inflammatory					
Yes	5 (0.82)	8 (1.32)			
No	601 (99.18)	598 (98.68)	0.62	0.20-1.91	0.40
Anticonvulsants					
Yes	0 (0.0)	2 (0.33)			
No	606 (100.0)	604 (99.67)	0.19	0.00-4.16	0.15
Antibiotics					
Yes	40 (7.25)	43 (7.20)			
No	511 (92.75)	554 (92.80)	1.00	0.64-1.57	0.97
Amoxicillin					
Yes	27 (4.72)	14 (2.37)			
No	546 (95.28)	578 (97.63)	2.04	1.06-3.93	0.02

(OR=1.3; 95% CI, 1.0 –1.6), and more strongly associated with bilateral CLP (1.7; 1.2–2.6), with a weaker association observed for CP.

Of 1,212 mothers, 996 (82.2%) was assessed for alcohol use in the first trimester of pregnancy, 416 (41.8%) cases and 580 (58.2%) controls. There was association between cases and controls (p=0,001), with greater proportion of cases (59 – 14.18%) than controls (45 – 7.75%) with alcohol use during the first trimester of pregnancy. In animal model, a study on eight pregnant mice provided with alcohol and cocaine found a significant increase in the number of dead fetuses and anomalies of the head such as hydrocephaly and complete orofacial craniofacial [25]. The evidence about alcohol consumption and NSCL/P was tenuous when the mother had not a 'binge' drinking patterns (high doses of alcohol in short periods of time). Likewise, the risk estimates were only minimally influenced after simultaneous adjustment for maternal cigarette smoking, race and ethnicity, education and multivitamin use [26,27].

The most commonly prescribed drugs in pregnancy, after prenatal vitamins, are antibiotics [28]. Among antibiotics, amoxicillin has been the preferred drug for the treatment of respiratory and urinary tract infections, conditions frequently encountered in women of childbearing age [15]. Thus, it is particularly important for clinicians, pregnant women, and public health officials to have information on the potencial teratogenic effects of amoxicillin in order to make risk-benefit judgments on its use early in pregnancy [12]. Amoxicillin can cross the placenta and could potentially influence fetal organogenesis [29], and the development of oral clefts [16].

In the present study, with respect to maternal use of amoxicillin, of 1,212 mothers, 1,165 (96.12%) was assessed, these, 573 (49.18%) cases and 592 (50.82%) controls. The proportion the mothers with amoxicillin use in the first trimester of pregnancy was greater in the cases (27; 4.72%) than in the controls (14; 2.37%). A significant association between maternal use of amoxicillin and oral cleft was detected (OR=2.04 [95% CI=1,06-3,93], p=0.02). Similarly, Lin *et al* (2012) [16] showed that maternal use of amoxicillin was associated with an increased risk of CL/P (adjusted OR=2.0 [95% CI=1.0-4.1]), with an OR of 4.3 (1.4-13.0) for third-gestational-month use. For CP, the OR for first-trimester amoxicillin was 1.0 (0.4-2.3), with an OR of 7.1 (1.4-36.4) for third-gestational-month use. In another study it was shown that major congenital malformations identified from computerized records were confirmed through medical record review. Overall, 869 (2.9%) of infants in the cohort had a confirmed major congenital malformation, with major malformations ranging from 2.5%-3.0% among the antibiotic-specific exposure groups [15].

Contradictorily to our results, other studies showed no association between amoxicillin and oral clefts. Results from the National Birth Defects Prevention Study did not focus on amoxicillin specifically and did not identify a positive association between penicillins and oral clefts [30]. Nielsen and Hviid (2012) [31] conducted a cohort study of 806,011 live births in Denmark from 1996 through September 2008. Overall, they didn't find antibiotic use in the first trimester to significantly increase the risk of NSCL/P. Czeizel *et al* (2001) [32] observed that treatments with ampicillin during the second and third months of gestation, which is the critical period for major congenital abnormalities, pose little if any teratogenic risk in human beings. Only a higher prevalence of isolated CP was found in the study and is considered as a hypothesis-generating findings. Limitations of our study were the relatively small number of patients that made use of amoxicillin. Studies with larger samples are needed to better understand the possible relationships between maternal use of amoxicillin and oral clefts.

Conclusion

In summary, our results found association between maternal smoking, alcoholism and use of amoxicillin in the first trimester of pregnancy and NSCL/P. The identification of modifiable risk factors for NSCL/P is the first step toward primary prevention. Further studies with a larger population are needed to confirm or exclude this possible association.

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