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ORIGINAL SCIENTIFIC ARTICLE

Nonsyndromic oral clefts and associated risk factors in the state of Bahia, Brazil

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Abstract

Aim Nonsyndromic cleft lip and/or palate (NSCL ± P) is the most common craniofacial birth defect. This study aims to determine demographic characteristics and the epidemiologic profile of NSCL ± P in Bahia, Brazil.

Subject and methods 692 patients of three Cleft Lip and Palate Treatment Centers were interviewed.

Results Cleft lip and palate (CLP) was the most frequent type of oral cleft (52.8%), particularly unilateral (34.8%) and affecting the left side ($p < 0.001$). Family history of cleft was found in 27.6%, especially between cousins (49.7%; $p < 0.001$). The Salvador metropolitan area represented 45.2% of the samples, followed by Mid-South (17.7%) and Mid-North Bahia (13.9%). In the South of the state, the risk of developing CL and CLP was statistically significant ($p = 0.03$; $p = 0.006$, respectively), and in the region of Vale do São Francisco there was a significant risk of developing CLP ($p = 0.01$), both in relation to CP. Young age and alcohol use in pregnancy were associated to giving birth to children with CLP ($p = 0.02$, $p = 0.03$, respectively). The use of folate and other vitamins diminished the risk of developing CL and CLP if compared to CP ($p = 0.009$).

Conclusion It is hoped that the results of this research may be useful in planning actions of public service that should take care of affected individuals.

Keywords Cleft lip · Cleft palate · Epidemiology · Etiology

Introduction

Nonsyndromic cleft lip and/or palate (NSCL ± P) are common developmental anomalies; however, prevalence worldwide is not completely known (Dixon et al. 2011; Leslie and Marazita 2013). Available data show that these malformations affect approximately one in every 500–2000 live births, depending on the geographic origin, ethnic group, gender and socio-economic pattern of the studied population (Dixon et al. 2011).

Some epidemiologic research has already been carried out in Brazil to determine orofacial cleft prevalence in several states, which data ranges from 0.36 to 1.54 per 1.000 live (Martelli-Junior et al. 2007; Rodrigues et al. 2009). The etiology and pathogenesis of the clefts, particularly nonsyndromic ones, are not widely known yet, despite all the efforts aimed at understanding these anomalies (Leslie and Marazita 2013). This fact reflects the complexity and variety of contributory mechanisms, as well as possible genetic and environmental factors that determine the development of these defects (Dixon et al. 2011; Aquino et al. 2014). Smoking (Little et al. 2002) and alcohol consumption (Boyles et al. 2010) by the mother have appeared as important risk factors to NSCL ± P during first the trimester of pregnancy. Different work activities with exposure to chemical agents have also been stated (Garcia and Fletcher 1998), though these factors are not always measurable. Polymorphisms of countless genes and loci in several populations showed association in etiology

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of NSCL ± P (Leslie and Marazita 2013; Aquino et al. 2014). Although multifactorial interactions are important in understanding this anomaly, the results still remain contradictory.

This research aimed to assess the characteristics of individuals with NSCL ± P in the state of Bahia, Brazil. It described the demographic and other characteristics of the sample, and assessed differences in the demographic profile of the patients in each of the cleft type groups. A description of associated predictive factors that are related to the cleft type was also performed, taking cleft palate (CP) as a reference.

Materials and methods

This research was approved by the Bahiana School of Medicine and Public Health Research Ethics Committee (378.066) and was performed in accordance with the ethical standards established in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Seven hundred and twenty-six patients and/or legal persons in 3 reference Cleft Lip and Palate Treatment Centers in the state of Bahia were interviewed, between the years of 2011 and 2015. The centers are placed in the Santo Antônio Hospital and, Martagão Gesteira Hospital, both in the city of Salvador and Esaú Matos Hospital, in the city of Vitória da Conquista. Informed consent was obtained from all individual participants included in the study.

The STROBE guidelines for reporting observational studies were used. On the first phase, 34 syndromic individuals were excluded from the study, totaling 692 patients. Adult individuals answered questionnaires and interviews were held with one or both parents of the children affected by NSCL ± P.

The questionnaire considered demographic (gender, place and date of birth) and familial features (mother's age at pregnancy, folic acid supplementation before and through the first trimester of pregnancy, smoking and alcohol consumption, illicit drug use, family history of cleft), cleft type and presence/absence of other health conditions.

The association of cleft type and life style features of patients, including mother's age at pregnancy, folic acid supplementation before and through the first trimester of pregnancy, smoking and alcohol consumption, and illicit drug use, were compared using x^2 or Fisher's exact tests. Multiple logistic regression analyses were performed to determine the adjusted relative risk (RR) with 95% confidence interval (CI) for the relationship between each feature and oral cleft while controlling for other key independent variables. Cleft palate (CP) was used as a reference. A p value ≤ 0.05 was considered statistically significant.

Results

Among the individuals interviewed, 49.4% were male and 50.6% were female. Ages varied from 1 month to 74 years, with an average of 12.7 years. CLP was the most frequent type of oral cleft (52.8%), with a preponderance of clefts being unilateral (unilateral 34.8%; bilateral 18.0%). CL was identified in 23.7% of interviewees (unilateral 18.0% and bilateral 4.7%), whereas CP was identified in 23.3%. Rare facial clefts represented 0.2% of the sample (Fig. 1).

In individuals with CL and CLP, the left side was more commonly affected (right 17.9%; left 31.0%; $p < 0.001$). Most patients were from urban areas (urban areas 73.8%; rural areas 24.8%; unanswered data 1.4%). Systemic alterations were present in 26.7% of the sample, in particular otolaryngological ones. Relatives with clefts were reported by 27.6% of those interviewees, notably between cousins (49.7%, $p < 0.001$).

Table 1 shows the distribution of NSCL/P in interviewees according to maternal characteristics. Maternal age at pregnancy and the frequency of alcohol, tobacco and illicit drug use during pregnancy were not significantly associated with cleft type ($p > 0.05$). CLP, regardless of the use of vitamin supplements, was the most frequent anomaly if related to other types of clefts ($p = 0.03$).

Although it is not shown in Table 1, the medications used more frequently during pregnancy were analgesics (26.3%), antibiotics (13.9%) and antiemetics (10.2%). Prior to conception as well as during pregnancy, 67.9% of mothers reported some medical conditions, in which hypertension (33.0%) and urinary infection (29.4%) were the most common.

The distribution of cleft types in macro-regions of the state showed statistic differences (Fig. 2 and Table 2). Salvador metropolitan area represented 45.2% of the sample, followed by Mid-South and Mid-North Bahia (17.7% and 13.9%, respectively). CLP had the highest prevalence in our samples from all regions, followed by CP and CL at similar frequencies. When comparing different regions of Bahia, the

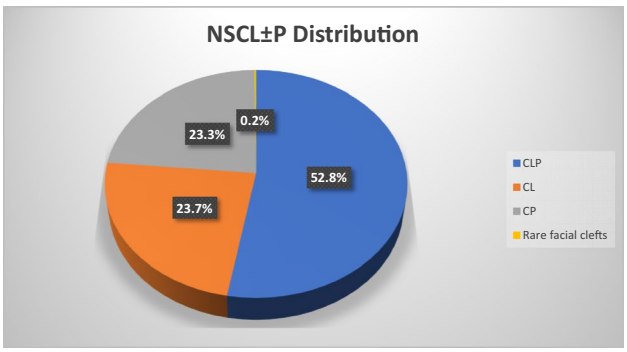

Fig. 1 NSCL ± P distribution

Table 1 Nonsyndromic cleft lip and/or palate distribution according to maternal characteristics

Variable	CL		CLP		CP		Total		*p*
	n	%	*n*	%	*n*	%	*n*	%	
Age of pregnancy (ages)									
15–25	23	28.7	40	50.0	17	21.3	80	11.5	
26–35	48	20.8	128	55.4	55	23.8	231	33.3	
> 36	79	24.6	167	52.0	75	23.3	321	46.3	0.108
Unanswered data	–	–	–	–	–	–	60	8.9	
Alcohol									
Yes	28	21.2	79	59.8	25	18.9	132	19.1	
No	123	24.1	267	52.3	121	23.7	511	73.8	0.292
Unanswered data	–	–	–	–	–	–	49	7.1	
Smoke									
Yes	14	19.4	40	55.6	18	25.0	72	10.4	
No	139	24.2	305	53.1	130	22.6	574	82.9	0.648
Unanswered data	–	–	–	–	–	–	46	6.7	
Ilicit drug									
Yes	1	10.0	5	50.0	4	40.0	10	1.4	
No	143	23.6	324	53.4	140	23.1	607	87.7	0.395
Unanswered data	–	–	–	–	–	–	75	10.9	
Supplementation									
No	24	21.4	70	62.5	18	16.1	112	16.2	
Folic acid	34	20.7	82	50.0	48	29.3	164	23.7	
Others	45	27.3	75	45.4	45	27.3	165	23.8	0.030*
Unanswered data	–	–	–	–	–	–	251	36.3	

*$P < 0.05$ Chi-square and Fisher's exact test

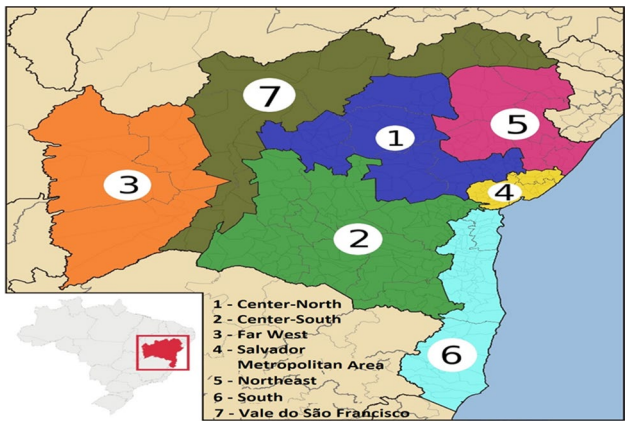

Fig. 2 Macro-regions in the state of Bahia, Brazil, according to Brazilian Institute of Geography and Statistics

greatest difference was between the South of the state and the Salvador metropolitan area.

Table 3 shows the distribution of cleft types in the most prevalent micro-regions of the state. Salvador represented 63.7% of the sample followed by the regions of Ilhéus and Itabuna (10.4%) and the city of Vitória da Conquista (10.0%). CLP was the most frequent cleft type in all areas with the exception of Vitória da Conquista, in which CL was more frequent (42.5%). The differences in distribution of cleft types between Salvador and Ilhéus/Itabuna, Santo Antônio de Jesus and Ilhéus/Itabuna were also described.

Table 4 shows the relative risk of CL and CLP related to CP adjusted for co-variants. Young maternal age (15–25 years) and maternal alcohol use were associated with given birth to children with CLP (RR 2.16,CI 95% 1.11–4.19, $p = 0.02$ and RR 1.54; CI 95% 1.07–2.38, $p = 0.03$, respectively). The use of folate and other vitamin supplements decreased the risk of CL and CLP in comparison to CP (folate: RR 0.44; CI 95% 0.23–0.82, $p = 0.009$; other vitamins and supplements: RR 0.43; CI 95% 0.22–0.80, $p = 0.008$). In the South of the state, the risk of being born with CL and CLP was statistically significant when compared to CP (CL: RR 3.56; CI 95% 1.20–10.56, $p = 0.03$; CLP: RR 3.81, CI 95% 1.49–15.19, $p = 0.006$). The risk was likewise statistically significant in the region of Vale do São Francisco to CLP (RR = 8.91; CI 95% 1.11–35.53, $p = 0.01$). Among micro-regions, Ilhéus/Itabuna cities showed a greater risk of developing of CL and CLP in relation to CP (CL: RR = 9.71, CI 95% 2.14–44.08, $p = 0.0005$; CLP: RR = 6.18; CI 95% 1.42–26.95, $p = 0.006$).

Table 2 Distribution of cleft types in macro-regions in the state of Bahia, Brazil

	Farwest		Vale do São Francisco Region		Mid-South		South		Mid-North		Northeast		Salvador metropolitan area		Total	
Cleft	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
CP	1.0	25.0	1.0	4.8	29.0	24.6	6.0	9.7	19.0	0.4	15.0	22.7	81.0	26.9	152.0	21.9
CL	1.0	25.0	4.0	19.0	35.0	29.7	20.0	32.3	17.0	18.3	13.0	19.7	64.0	21.3	154.0	23.2
CLP	2.0	50.0	16.0	76.2	54.0	45.8	36.0	58.1	57.0	61.3	38.0	57.6	156.0	51.8	359.0	54.0
Total	4.0	0.6	21.0	3.1	118.0	17.7	62.0	9.3	93.0	13.9	66.0	9.9	301.0	45.2	665.0	96.9
Unanswered data	–		–		–		–		–		–		–		27.0	3.1

Table 3 Distribution of cleft types in the most prevalent micro-regions in the state of Bahia of Bahia, Brazil

Cleft	Salvador		Ilhéus/itabuna		Vitória da Conquista		Santo Antonio de Jesus		Jequié	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%
CP	68	26.5	2	4.8	11	27.5	11	31.4	4	13.8
CL	56	22.1	16	38.1	17	42.5	6	17.2	6	20.7
CLP	132	51.4	24	57.1	12	30.0	18	51.4	19	65.5
Total	256	63.7	42	10.4	40	10.0	35	8.7	29	7.2

Discussion

This study evaluated the cleft population profile and associated risk factors in the state of Bahia, Brazil. The study of genetic markers in different populations is important in identifying both common and population-specific risk factors. This is particularly relevant considering the ethnically mixed Brazilian population, in which individuals commonly have variable levels of Amerindian, European and African ancestry (Salzano and Sans 2014). Historically the state of Bahia received the largest number of African immigrants, mostly Sub-Saharan, and remains the state with the highest proportion of African genomic ancestry (Borges et al. 2015).

There was no statistically significant difference in gender among the patients with NSCL/P observed in this study. However, other studies performed in Spain (Garcia and Fletcher 1998), Denmark (Hagberg at al. 1998), Croatia (Magdalenic and Bagatin 2005), the Philippines (Murray et al. 1997) and Iran (Mirfazeli et al. 2012) found a higher frequency in males. In Brazil, studies with populations from other states also identified a higher frequency of NSCL/P in male (Martelli-Junior et al. 2007; Rodrigues et al. 2009; Martelli et al. 2012; Campos Neves et al. 2016). Regarding the cleft side, unilateral (52.8%) was more frequent than bilateral (22.7%). The existing literature likewise converges on a higher incidence of unilateral clefts, supporting the findings of the current study (Reddy et al. 2010). In the current study the left side was more frequently affected than the right. Such evidence is also supported by literature (Magdalenic and Bagatin 2005). Preferential distribution of clefts throughout the left side sustains the hypothesis that in the initial phases of fetal development, there may be an asymmetric expression of genes belonging to nodal signaling pathways. In vertebrate embryos, such genes are expressed predominantly on the left side (Genisca et al. 2009). Club foot and microtia show predilection on the right side, whereas CLP and congenital hip dysplasia show a predilection for the left (Paulozzi and Lary 1999).

Family history of cleft is a consistent finding, highlighting the genetic influence in the development of NSCL/P (Kot and Kruk-Jeromini 2007). In our study, 31.0% of cases reported a family history orofacial cleft, very similar to the percentages reported by Martelli et al., (35% reporting family history of NSCL/P), whose study was also performed in Brazil (Martelli et al. 2010). These authors also observed a high frequency between cousins, as in our study.

For some genetic diseases, advanced maternal age is considered a risk factor, but for NSCL/P evidence remains divergent (Kot and Kruk-Jeromini 2007). In California, women older than 39 years were observed to have twice the risk of having a child with either type of cleft in comparison to mothers between ages 25 and 29 (Shaw et al. 1991). In our study there was a higher frequency of mothers aged 36 years of age and older (50.8%) compared with mothers between ages 15 and 25, with the risk of giving birth to a child with CLP in the former twice as high (RR 2.16, CI 95% 1.11–4.19, $p = 0.02$). In a recent meta-analysis, no

Table 4 Risk of developing CL(Cleft Lip) and CLP (Cleft Lip and Palate) related to CP (Cleft Palate)

Maternal variable	CL × CP			CLP × CP		
	RR	CI 95%	*p*	RR	CI 95%	*p*
Age						
15–25	1.03	0.50–2.13	0.93	2.16	1.11–4.19	0.02*
26–35	0.95	0.45–197	0.89	1.53	0.78–2.99	0.21
> 36	1.00	–		1.00	–	
Alcohol						
Yes	1.25	0.51–3.07	0.61	1.54	1.07–2.38	0.03*
No	1.00	–		1.00	–	
Smoke						
Yes	0.61	0.17–2.12	0.44	0.94	0.52–1.71	0.86
No	1.00	–		1.00	–	
Supplementation						
No	1.00	–		1.00	–	
Folicacid	0.69	0.37–1.26	0.22	0.44	0.23–0.82	0.009*
Others	0.68	0.66–1.54	0.08	0.43	0.22–0.80	0.008*
Macro-region						
Farwest	1.33	0.02–15.14	0.43	1.01	0.06–15.38	0.97
Vale S. Francisco	5.81	0.19–20.58	0.28	8.91	1.11–35.53	0.01*
Center-South	1.54	0.96–2.73	0.13	0.98	0.68–1.43	0.94
South	3.56	1.20–10.56	0.03*	3.81	1.49–15.19	0.0006*
Center-North	1.15	0.51–3.07	0.78	1.73	0.83–3.58	0.14
Northeast	1.13	0.38–3.37	0.80	1.58	0.85–2.97	0.35
Salvador	1.00	–		1.00	–	
Micro-region						
Jequié	1.82	0.48–6.77	0.36	2.44	0.80–7.48	0.10
Ilhéus/Itabuna	9.71	2.14–44.08	0.0005*	6.18	1.42–26.95	0.0006*
S. Antonio de Jesus	0.66	0.23–1.90	0.44	0.84	0.87–1.88	0.68
Vit. da Conquista	1.97	0.81–4.33	0.13	0.56	0.23–1.34	1.19
Salvador	1.00	–		1.00		

*$p < 0,05$ Chi-square and Fisher's exact test

association between early maternal and paternal age and the occurrence of oral clefts was observed (Herkrath et al. 2011).

In current literature, many agents have been associated with congenital abnormalities. Although alcohol is considered the most consumed teratogen in the world, an association between maternal alcohol consumption and NSCL/P is still conflicting (Romitti et al. 1999; DeRoo et al. 2008). In our study, we found that this chemical agent, when ingested by mothers, was a risk factor for CLP (RR 1.54, CI 95% 1.07–2.38, $p = 0.03$). Regarding genetic susceptibility and alcohol consumption, Romitti et al. (1999) studied the presence of allelic variants of the *MSX1* gene. MSX1 is intensely expressed during facial development, and consumption of only one alcoholic beverage per week by the mother was shown to correlate with a rise in NSCL/P risk, with a more significant risk in particular allelic variants in *MSX1*. In a case–control study performed in Norway, mothers who consumed five or more glasses of alcoholic beverages during the first trimester of gestation presented with twice the risk of developing of oral clefts in their children. These results were more evident in mothers and children with the *ADH1C* haplotype associated with reduced alcohol metabolism (Boyles et al. 2010).

Ingestion of alcoholic beverages is often associated with exposure to other teratogenic agents such as tobacco (Krapels et al. 2006). Studies about maternal smoking in etiology of the NSCL ± P are also contradictory. In a meta-analysis including 24 studies, a statistically significant association was found between maternal smoking and NSCL/P and CP (Little et al. 2002). The present study observed only 10.4% of the mothers using tobacco during pregnancy, and no association between tobacco use and CL and CLP when compared with CP.

Regarding vitamin supplements, 47.53% of the mothers reported the use of folate or a multivitamin either before conception or in the first trimester of gestation. A drop in the risk of CL and CLP compared to CP was

observed in those who used folate and other supplements. Even with conflicting data in the existing literature, the importance of folate to prevent NSCL/P has been widely discussed. Recent study has shown genetic variations that influence folate absorption, transportation and metabolism for a better understanding of its function on etiology of the NSCL ± P (Aquino et al. 2014). Aquino et al. (2014) evaluated the association of *MTHFR* and *MTHFD1*polymorphisms with the development of NSCL ± P, with results showing that the presence of the rs2274976 A allele increases the risk of such anomalies in the Brazilian population.

The state of Bahia, located in the northeast region of Brazil, is divided into seven macro-regions, of which two, the South and Vale do São Francisco, were related to a higher risk of clefts. However, this association is not truly representative, since the number of nonsyndromic oral cleft patients in these regions was low. A possible explanation for this finding is that patients from certain distant regions faced difficulties in attending the reference centers for oral clefts treatment which are located in the eastern part of the state. In fact, there are geographic differences regarding the socioeconomic conditions of each region that may impact mainly on the patient's displacement to the references centers closer to their homes.

The present study has some limitations that should be highlighted. As it is an exploratory study that analyzed a database, the authors do believe in the possibility of information bias, especially considering the age variability among the individuals. Only patients accompanied by their parents answered the questionnaire completely. Adult patients could not describe maternal and paternal habits properly. For this reason, we have lost data regarding some variables. To avoid selection bias, we included both adults and infants and all the variables included in the study were considered. It is important to point out that the study's sample was a convenience one and all the participants with oral clefts that looked for specialized treatment and went to the reference centers were considered.

The estimated prevalence of 1: 1000 does not refer to the state of Bahia, but to our country, Brazil. As a matter of fact, the estimated population in the state of Bahia is 15,344,447 (2017) and considering a prevalence of 1: 1000, we could estimate more than 15,000 individuals with CL/P. Certainly, the population with nonsyndromic cleft lip and/or palate in the state of Bahia state is larger than the sample represented in the study. In our state, there are only three reference centers for the treatment of craniofacial defects, to which the patients in the state are usually referred for a multidisciplinary approach. Although this population does not represent the entire group of patients with nonsyndromic cleft lip and/or palate in the state, it is significant because it expresses a specific and directed demand for the care of these individuals. The patients who arrived at the three centers were directed to the team of researchers by free demand.

The micro-regions of Ilhéus and Itabuna, located in the South of Bahia, showed a higher risk in developing CL (OR 9.71, CI 95% 2.14–44.08, $p = 0.0005$) and CLP (OR 6.18, CI 95% 1.42–26.95, $p = 0.006$) when compared to CP. However, inferences about these regions should be made with caution. There is a chance that genetic contributions influence these results, since genes responsible for correct facial development are considerably susceptible to associated factors.

In general, we are still unaware of the existence of possible genetic determinants that could contribute to the occurrence of CL in the macro and micro regions studied. The state of Bahia, located in the northeastern region of Brazil, has an average per capita income of 200 dollars and many municipalities are in a situation of social vulnerability. Some aspects such as transportation difficulties, undernutrition, illiteracy, consanguinity may have interfered with the risk analysis so that these results should be interpreted with caution.

Although this study was the first one to attempt to characterize the population of patients with NSCL/P in the state of Bahia, it has some limitations. The study considered only some factors that are associated to oral cleft development. Additional case control studies have to be performed to describe the role of these associated factors thoroughly. Despite such limitations, this study reinforces the importance of epidemiological surveys tracing the profile of users of oral cleft reference centers. Such surveys should also cover important environmental aspects to identify associated factors that influence NSCL/P development in other states of Brazil. A comprehensive understanding of these factors, combined with an understanding of the etiology of the oral cleft, will allow the development of strategies seeking its early prevention.

Conclusion

Although this study did not recruit the totality of patients with NSCL/P in the state of Bahia, it may contribute to a better characterization of this population and add knowledge about possible factors associated with the development of oral cleft in Brazil, a country whose population is ethnically mixed and diverse.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional human research committee of the Bahiana School of Medicine and Public Health with reference number 378.066, and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in this study.

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