Oral clefts, consanguinity, parental tobacco and alcohol use: a case-control study in Rio de Janeiro, Brazil

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Abstract: This hospital-based, case-control study investigated the possible associations between family history of malformations, parental consanguinity, smoking and alcohol drinking and nonsyndromic orofacial cleft (OC, subdivided in 2 main groups: CL/P - cleft lip with or without cleft palate and CP - cleft palate alone). 274 cases were matched (age, sex and place of residence) to 548 controls. Odds ratios (OR) and 95% confidence intervals (95% CI) – adjusted for maternal age, schooling and smoking / alcohol use – were calculated by conditional logistic regression. The results demonstrated that the history of oral clefts either in the father's (CL/P: OR = 16.00, 5.64-69.23; CP: OR = 6.64, 1.48-33.75) or in the mother's family (CL/P: OR = 5.00, 2.31-10.99, CP: OR = 12.44, 1.33-294.87) was strongly associated with both types of clefts, but parental consanguinity was associated only with CL/P (OR = 3.8, 1.27-12.18). Prevalence of maternal smoking during the first trimester of pregnancy was higher among cases but the OR (1.13, 0.81-1.57) was not statistically significant. Maternal passive smoking (nonsmoking mothers) during pregnancy was associated with CL/P (1.39, 1.01-1.98) but not with CP. Maternal alcohol use during the 1st trimester increased odds for CL/P (OR = 2.08, 1.27-3.41) and CP (OR = 2.89, 1.25-8.30), and odds for OC tended to increase with dose. Neither smoking nor alcohol use by fathers increased risks for OC. This study provides further evidence of a possible role of maternal exposure to tobacco smoke and alcohol in the etiology of nonsyndromic oral clefts.

Descriptors: Cleft lip; Cleft palate; Ethanol; Tobacco; Consanguinity.

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Introduction

Clefts of the lip (CL), without or with cleft palate (CL/P), as well as isolated cleft palate (CP) are among the most common congenital anomalies, occurring approximately once per 1,000 live births among Caucasians.[1,2] In South America, the authors[3] found a prevalence as high as 0.87 per 1,000 for CL/P and as high as 0.13 per 1,000 for CP in 56 hospitals of 8 countries between 1967 and 1981. The prevalence of oral clefts (CL/P plus CP) in Brazil was estimated to be 0.19 per 1,000 live births in the period of 1974 - 1994, with 74% of CL/P and 26% of isolated CP.[3]

The etiology of nonsyndromic oral clefts remains to be completely understood, but today's best evidence suggests that these birth defects are multifactorial in origin with both genetic and environmental causative factors.[4]

The present study was undertaken to examine whether parental smoking and alcohol use during the first trimester of pregnancy increase the risk for nonsyndromic orofacial clefts in the offspring. In addition, we also provided data on the association of family history of birth defects and parental consanguinity with the occurrence of oral clefts.

Material and Methods

A hospital-based case-control study was performed in the city of Rio de Janeiro. Cases were defined as infants with 0-24 months of age presenting cleft lip, without (CL) or with cleft palate (CL/P), or cleft palate alone (CP), not associated with any other birth defect or syndrome (i.e. nonsyndromic oral clefts). All cases were patients from the Nossa Senhora do Loreto Municipal Hospital, a reference pediatric unit for orofacial clefts. Controls were infants without any congenital anomaly admitted to the hospital to treat different clinical abnormalities. Control infants were selected among patients admitted to the same hospital or, when this was not feasible, to another pediatric hospital located in the same county or state geographic region as that of the cases' residence. Proven or suspected cases of syndromic malformations were excluded from controls, thus resulting in a study consisting of 274 cases and 548 controls. These controls were selected and matched to each case according to sex, age (± 2 months), and city or state region of the parent's residence.

The research protocol was reviewed and approved by the Ethical Committee, National School of Public Health, FIOCRUZ. The questionnaire used in this study was based on a standard questionnaire for gathering data on environmental and occupational exposures validated by the International Agency for Research on Cancer (IARC).[5] The interview provided information on the parents' sociodemographic characteristics, age and place of residence, as well as consanguinity,[6] family history of malformations, mother's medical history, maternal use of drugs, parent's alcohol and tobacco use including passive smoking. Odds ratios (OR) and respective 95% confidence intervals (95% CI) for the magnitude of associations between the studied variables and oral clefts were estimated by conditional logistic regression using EGRET software (Cytec Software Corp: Statistics and Epidemiology Research Corporation, Seattle, WA, USA). The univariate and bivariate analyses evaluated the possibility of confounding factors when the OR by stratum presented a variation greater than 20% in relation to the gross OR, as well as interaction, using Wolff's chi-square test.[7] Whenever a confounding factor was observed, a Mantel-Haentzel stratified analysis was performed to evaluate risk trends. Means were compared using Tukey's test.[7] The regression analyses controlled for the variables maternal age, since the literature reports an increase in the probability of congenital malformations associated with increased age, and maternal schooling, which did not show significant differences between cases and controls in most strata but did present a difference for secondary schooling.

In the same way, alcohol and tobacco use was controlled respectively, since the related literature shows an interaction between these two variables. The modeling criterion adopted was that of biological plausibility, using the enter method and admitted variables with p-value < 0.25 in the chi-square test in the model.[7]

In this study, the goal was to use the resulting regression equation to identify variables that best ex-

plain the level of the dependent variable – a descriptive or exploratory purpose.[8] Only co-variables that had presented potential interaction were included in the logistic regression.

Results

General characteristics of the sample

The general characteristics of the sample showed that, except for a higher proportion of mothers with complete intermediate education (including high school years) among cases (19.7% *versus* 11.8%, $p < 0.003$), there were no other major differences in maternal schooling between cases and controls (data not shown). In this study, cases of CL/P were associated with gestational ages at delivery of less than 37 weeks as well as of more than 42 weeks. No difference of weight and height at birth between cases of orofacial clefts and control patients was found in the present study. The gestational age at delivery (by the Capurro method) was less than 37 weeks in 23.2% of the whole sample (cases and controls), and more than 42 weeks in the remaining 8.7%. Cases of CL/P were associated with < 37 weeks as well as with > 42 weeks of gestational age with odds ratios (95% CI) as high as 2.86 (1.35-3.03) and 5.56 (1.54-6.25), respectively. Weight ($p = 0.08$) and length ($p = 0.46$) at birth did not differ significantly between cases and controls.

Preliminary analysis

Tables 1 and 2 show estimated odds ratio for history of congenital anomaly in the family and consanguinity and parental habits (tobacco and alcohol exposure) in association with these congenital anomalies. In the bivariate analysis, the variables that measured congenital anomaly did not present any interaction effect with other co-variables.

Logistic regression analysis - parental smoking and alcohol drinking

The odds ratio for maternal smoking during one year before pregnancy – adjusted for mothers' schooling, age and alcohol consumption – was 1.59 (95%CI = 1.04-2.44) for CL/P and 0.82 (95% CI = 0.34-1.79) for CP. For maternal smoking during the first trimester of pregnancy, no significant increase in risk was found for both types of orofacial cleft. An increase in risk according to duration of smoking habit was found for CL/P ($p = 0.03$) and for all types of clefts ($p = 0.02$), but not for CP. Risk increased according to the number of cigarettes smoked per day – during the first trimester of pregnancy – for CL/P ($p = 0.03$) and for all types of clefts ($p = 0.03$), but not for CP. For CP, an increase in risk according to duration of smoking habit was identified in the univariate analysis, but not in the

Table 1 - Univariate analysis for history of congenital anomaly in the family (CA) and consanguinity in association with orofacial clefts (OC), Rio de Janeiro, 2005.

		Number (N)[a]			Odds ratio (CI 95%)		
		CL/P	CP	Controls	CL/P	CP	OC
History of CA	Without CA in the family[b]	103	43	210	1.00 –	1.00 –	1.00 –
	OC in the paternal family[b]	33	8	9	16.00 (5.64-69.23)	6.64 (1.48-33.75)	14.01 (6.32-32.01)
	OC in the maternal family[b]	26	5	27	5.00 (2.31-10.99)	12.44 (1.33-294.87)	5.96 (3.03-11.84)
	OC in both families[b]	42	9	77	2.86 (1.61-5.00)	1.06 (0.41-2.70)	2.04 (1.33-11.84)
History of kinship between parents[c]	Absent	198	65	531	1.00 –	1.00 –	1.00 –
	Present	10	1	10	3.80 (1.27-12.18)	0.50 (0.02-3.98)	3.67 (0.86-6.35)

[a]excludes indeterminate responses; [b]CA = congenital anomaly, excepting histories of mental retardation; [c]considering close or first degree relatives as defined in the ECLAMC (Latin American Colaborative Study of Congenital Malformations): blood relatives such as first-degree cousins, uncle/nephew; aunt/niece.

Table 2 - Univariate analysis for estimated odds ratio for oral clefts by parental tobacco and alcohol exposure, Rio de Janeiro, 2005.

		Cases N (%)[a]	Controls N (%)[a]	Odds ratio[ac] (CI 95%)		
				CL/P	CP	OC
Maternal smoking habits	Nonsmoking mothers	206 (75.20)	454 (82.90)	1.00 –	1.00 –	1.00 –
	Maternal smoking in the year previous to gestation	68 (24.80)	94 (17.10)	1.50 (1.07-2.16)	0.92 (0.54-1.59)	1.32 (0.93-2.01)
	Maternal smoking status during 1st trimester	51 (18.60)	88 (16.10)	1.23 (0.82-1.76)	0.52 (0.21-1.15)	1.13 (0.81-1.57)
Duration of the exposure	≤ 10 years	44 (64.70)	63 (64.30)	0.51 (0.17-1.85)	0.42 (0.05-5.32)	0.47 (0.16-1.43)
	> 10 years	24 (35.30)	35 (35.70)	2.34 (1.24-3.86)	0.49 (0.12-1.87)	1.72 (0.93-2.83)
	significance test			p = 0.03	ns	p = 0.02
Number of cigarettes/day[b]	≤ 10	31 (60.8)	55 (62.50)	0.42 (0.15-1.31)	0.52 (0.53-4.40)	0.42 (0.14-1.15)
	> than 10	20 (39.20)	33 (37.50)	1.75 (0.94-3.27)	0.47 (0.07-1.64)	1.32 (0.73-2.45)
	significance test			p = 0.03	ns	p = 0.03
Passive maternal tabagism[c]		166 (60.60)	281 (51.00)	1.41 (1.12-2.01)	1.69 (0.87-3.13)	1.51 (1.13-2.11)
Paternal tabagism	in the year previous to gestation index	68 (24.90)	183 (23.20)	1.31 (0.77-1.87)	0.52 (0.22-1.27)	1.29 (0.91-1.61)
	During 1st trimester	59 (21.60)	118 (21.40)	1.17 (0.80-1.75)	0.58 (0.19-1.27)	1.02 (0.75-1.52)
Maternal alcohol intake	Absent	201 (73.4)	467 (85.3)	1.00 –	1.00 –	1.00 –
	Present in the year previous to gestation	73 (26.6)	81 (14.7)	2.11 (1.47-3.01)	3.92 (1.75-8.72)	2.12 (1.47-3.22)
	1st trimester	51 (18.7)	53 (9.6)	2.16 (1.42-3.27)	2.91 (1.28-8.34)	2.17 (1.40-3.39)
Duration of the exposure	≤ 6 years	9 (13.1)	6 (7.6)	1.28 (0.36-4.10)	0.53 (0.08-4.82)	0.98 (0.94-1.07)
	> than 6 years	64 (86.9)	75 (92.4)	1.68 (0.35-7.61)	2.27 (0.69-7.42)	2.31 (1.41-3.82)
	significance test			ns	ns	p = 0.05
Amount (g) of alcohol/day	≤ 96 g	8 (16.1)	4 (7.6)	1.07 (0.35-3.41)	1.30 (0.24-7.73)	0.97 (0.91-1.04)
	> than 96 g	43 (83.9)	49 (92.4)	2.06 (0.97-4.26)	2.03 (0.74-5.51)	1.92 (1.12-3.27)
	significance test			ns	ns	p = 0.05
Frequency of occasional consumption *versus* daily		(95.7 x 4.3)	(95.0 x 5.0)	2.69 (1.03-7.95)	1.44 (0.38-5.90)	1.19 (0.25-7.11)
Paternal alcohol intake absent *versus* present		(47.4 x 52.6)	(50.1 x 49.9)	1.07 (0.72-1.48)	1.45 (0.82-1.37)	1.16 (0.85-1.57)

[ac]calculated OR having as reference the absence of the risk factor; [a]excluded undetermined answers; [b]tabagism during the first trimester of gestation; [c]reported as tabagism in the home and/or work environments and attributed to non-smokers.

logistic regression. Maternal passive smoking was also associated with CL/P.

As shown in Table 3, the odds ratios for maternal alcohol intake during one year before pregnancy – adjusted for mother's schooling, age and tobacco smoking – was 1.80 (95%CI = 1.40-2.84) for CL/P and 3.87 (95% CI = 1.73-8.65) for CP. Maternal alcohol consumption during the first trimester of pregnancy was also associated with CL/P and with CP. Analysis of categories for duration of maternal drinking habit did not show any significant association except for more than 6 years of alcohol use and all types of oral clefts. Categories for alcohol consumption did not show any significant association, except for drinking more than 96 g of ethanol per day and all types of oral clefts (OR = 1.88, 95% CI = 1.10-3.20). The proportion of mothers reporting a daily intake of alcohol during the first trimester (approximately 5%) did not differ between cases of all oral clefts and controls. Maternal daily drinking during pregnancy, however, was more frequent among cases of CL/P than among controls (OR = 2.67, 95% CI = 1.00-7.91).

Discussion

Since mothers of malformed children apparently recall exposures during pregnancy more accurately than healthy control's mothers, recall bias has been a cause for concern in any restrospective study of risk factors for congenital anomalies.[9] The use of malformed controls, however, has also been questionned because it implies that the specificity of the association – and not the association itself – is being examined. Although we can not rule out the occurrence of a recall bias, it seems very unlikely in the current study for two reasons. Firstly, because affected infants (cases of nonsyndromic oral clefts) were compared to non-malformed sick controls admitted to the hospital. Secondly, because chronic maternal exposures such as smoking and alcohol intake, are unlikely to be strongly influenced by recall deficits.

Table 3 - Adjusted odds ratio by logistic regression of relative tobacco exposure and alcohol intake (year previous to gestation index and 1st trimester of gestation) in association with orofacial clefts (OC), Rio de Janeiro, 2005.

		Odds ratio[ac] (CI 95%)[b]		
		CL/ P	CP	OC
Maternal smoking habits[b]	Nonsmoking mothers	1.00 –	1.00 –	1.00 –
	Maternal smoking during year previous to gestation	1.59 (1.04-2.44) p = 0.001	0.82 (0.34-1.79) p = 0.08	1.28 (0.87-1.97) p = 0.06
Duration of the exposure > 10 years		2.12 (1.18-3.80) p = 0.03	0.47 (0.10-1.84) p = 0.28	1.70 (1.07-2.97) p = 0.02
Passive maternal tabagism		1.39 (1.01-1.98) p = 0.001	1.67 (0.90-3.11) p = 0.18	1.48 (1.09-2.01) p = 0.001
Maternal alcohol intake[c]	Absent	1.00 –	1.00 –	1.00 –
	Present in the year previous to gestation	1.80 (1.40-2.84) p = 0.001	3.87 (1.73-8.65) p = 0.001	2.09 (1.42-3.09) p = 0.001
	1st trimester	2.08 (1.27-3.41) p = 0.001	2.89 (1.25-8.30) p = 0.001	2.16 (1.39-3.35) p = 0.001
Duration of the exposure (years) > than 6 years		1.65 (0.37-7.58) p = 0.58	2.24 (0.67-7.38) P = 0.82	2.29 (1.38-3.79) p = 0.03
Amount (g) of alcohol/day > than 96 g		2.05 (0.98-4.28) p = 0.36	2.01 (0.71-5.49) p = 0.67	1.88 (1.10-3.20) p = 0.03
Frequency of occasional consumption *versus* daily		2.67 (1.00-7.91) p = 0.04	1.40 (0.33-5.84) p = 0.27	1.18 (0.21-7.14) p = 0.48

[ac]calculated OR having as reference the absence of the risk factor; [a]excluded undetermined answers, [b]odds ratio adjusted for educational level, age and maternal alcohol intake; [c]odds ratio adjusted for educational level, age and maternal tabagism.

Family aggregation of cases of oral clefts has been described and a 10-fold increase in risk to siblings of cases compared to siblings of controls was described.[1] In the same line, our data also showed that parental consanguinity was significantly associated with CL/P but not with CP only. Bias, lack of statistical power, and population diversity can explain the diverse studies' results. In the aggregate, transforming growth factor alpha gene (TGFA) is probably a genetic modifier of clefting in humans, which is consistent with the oligogenic model suggested for nonsyndromic oral clefts.[10]

A meta-analysis on maternal exposure to tobacco smoke was also published.[4] Of 11 studies included in the meta-analysis, a significant association of maternal smoking with oral clefts was found in approximately half of them, *i.e.* in 5 (4 with CL/P and 1 with CP alone). The meta-analysis overall estimated risk for maternal cigarette smoking during the first trimester of pregnancy was 1.29 (95% CI = 1.18-1.42) for CL/P and 1.32 (95% CI = 1.09-1.60) for CP alone. A review study[11] found an association between maternal smoking during pregnancy either with isolated CL/P or with CP alone. However, it also found a significant positive dose-response for maternal smoking among infants with CL/P plus other birth defects. Lorente *et al.*[12] (2000) reported an increased risk (OR = 1.79, 95% CI = 1.07-3.04) for all cases of CL/P, including those cases of clefts in patients with malformations other than clefts, but not for isolated clefts (CL/P and CP alone), associated with maternal smoking during the first trimester of pregnancy. The literature reported that tobacco use during pregnancy was significantly more frequent among mothers of cases of nonsyndromic cleft lip and/or palate than among control's mothers (OR = 1.43, 95% CI = 1.25-1.64).[13]

Altogether, the foregoing published reports and the results from the present study seem to suggest that maternal smoking during pregnancy increases the risk for nonsyndromic oral clefts, but the strength of this association seems to be relatively weak. Our results are thus at variance with those reported by Beaty *et al.*[1] (2001) who did not find any significant association of maternal passive smoking with oral clefts after adjustment for maternal age and education.

The results of previous studies on the possible association between maternal alcohol use during pregnancy and risk of oral clefts in the offspring have been inconsistent. Lorente *et al.*[12] (2000) identified an increased risk for isolated CP, but not for CL/P, among mothers who drank during the first trimester of pregnancy (OR = 2.99, 95% CI = 1.38-6.45). This increase in risk, however, was not dose related since it was seen in the offspring of mothers who drank less than 70 g of ethanol per week but not among children from those who drank 70 g or more. Spilson *et al.*[13] (2001), on the other hand, found that maternal alcohol use during pregnancy among oral cleft cases was slightly more frequent than among controls. Our results also indicated that the risk for oral clefts (CL/P plus CP) tended to increase with a daily dose of ethanol and the risk for CL/P tended to increase with frequent drinking.

Conclusions

In light of the results obtained here, we concluded that the potential effect of prolonged maternal smoking during pregnancy increases the risk for nonsyndromic oral clefts, and that there is a tendency to increase the risk for CL/P with a daily dose of ethanol and with frequent drinking. Some limitations to this type of exploratory investigation can be identified, like the small number of observations in each *stratum*, which sometimes hinders the construction of risk measures or generates estimates with wide confidence intervals.

The current study provided evidence for strong associations between oral clefts and a family history of malformations and parental consanguinity. It also provided a moderate, consistent and statistically significant association between oral clefts and alchool intake during the first trimester of gestation and, to a lesser extent, maternal exposure to tobacco during a year previous to gestation.

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