Influence of Parental Exposure to Risk Factors in the Occurrence of Oral Clefts

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ABSTRACT

Statement of the Problem: Non-syndromic cleft lip and palate are the most frequent craniofacial abnormalities in humans. The genetic, environmental and behavioral factors involved in this malformation must be clarified in different parts of the globe in the view of implementing preventive measures.

Purpose: To analyze the influence of parental exposure to risk factors on the occurrence of oral clefts.

Materials and Method: A case-control study was conducted with 150 mothers of oral cleft children paired by the children’s gender to 300 mothers of children without congenital anomalies from Mato Grosso, Brazil, for the study of the variables: gender and race/color of the children; parental educational level; age; number of pregnancies; prenatal care; obesity; stress; diabetes; hypertension; use of medications, alcohol and illicit drugs; smoking and exposure to ionizing radiation during the first trimester of pregnancy. The results were analyzed in relation to the chances possibility of each variable for the occurrence of oral cleft through the bivariate and multivariate analysis by applying the model of logistic regression.

Results: Passive smoking, obesity, exposure to ionizing radiation and use of antibiotics were associated with the presence of clefts. The use of folic acid and analgesics were identified as preventive factors. The father’s low educational level was found as a risk factor, while the black race/color was a preventive factor; nevertheless these variables were not associated in the multivariate analysis.

Conclusion: The results reinforce the need to follow the pregnant women, especially in the first trimester of pregnancy, in order to control the identified risk factors.

Clinical Relevance: Knowing the factors associated with the occurrence of oral clefts and those associated with its prevention, it is possible to apply specific health promotion measures during pregnancy, which can result in the reduction of oral clefts’ occurrence.

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issue [4]. Maternal exposure to risk factors in the first trimester of pregnancy has been associated with the occurrence of orofacial clefts because of the interference caused in the fusion of the craniofacial processes that form the primary and secondary palates, involving the lip, alveolar process, hard palate and soft palate, between the fourth and the twelfth week of pregnancy. As between the 6th and the 8th week of gestation the fusion of the upper lip is completed and between the 8th and 12th week the hard and soft palate’s fusion are completed, interferences in this period may lead to cleft lip, cleft palate and cleft lip and palate [5]. Aesthetic and functional problems caused by oral clefts require long-term treatment. They represent a higher risk of morbidity, difficulty in feeding, changes in speech and hearing, affecting the social interaction of the individual and cause psychological and financial impact in their families [1-5].

Studies aiming to understand the interaction between genetic, environmental and behavioral factors involved in this malformation in early pregnancy have been made in the view of implementing preventive measures [3-4]. Among the environmental and behavioral factors associated some relevant ones are smoking [5-7], alcohol consumption [5, 8-9], birth order, birth interval [10], folic acid deficiency [11-12], parental age [8, 11-12], race/color of skin [13-15], diabetes, hypertension [16-18], use of medications [8, 19] and exposure to ionizing radiation [20-21]. It is possible to intervene in these factors with health promotion measures particularly during pregnancy, which can result in reduction of oral clefts’ occurrence.

This study aims to analyze the influence of parental exposure to risk factors on the occurrence of oral clefts; a priority for the advancement of research on the field [4].

Materials and Method
A case-control study was conducted with genitors of the State of Mato Grosso, Mid-West Brazil. A total of 878 medical records of patients undergoing treatment at the Oral Clefts Rehabilitation Service of the General Hospital of the University of Cuiabá were analyzed for the composition of the case group. Among them, the mothers of children were selected according to the following inclusion criteria: presenting non-syndromic isolated cleft lip and/or palate; the pregnancy period occurred in the State of Mato Grosso; being under six years old. From the 200 progenitors of children who met the inclusion criteria, 150 attended the hospital on the dates scheduled to participate in the study.

The control group was consisted in the proportion of two controls for each case paired according to the sex of the children in the case group, totaling 300 genitors in the group. These control group mothers where enrolled in the University General Hospital (138 progenitors) and Santa Helena Hospital (212 progenitors). Both institutions constitute the State Hospital Reference System for the High Risk Pregnancy Assistance in Cuiabá. It was established as inclusion criterion being the mother of newborns without malformations detected at birth and as exclusion criterion the pregnancy not occurred in Mato Grosso. Therefore, the entire study population consisted of 450 genitors.

A structured questionnaire was used for data collection. It had questions about the child, the parents and maternal exposure to risk factors in the first trimester of pregnancy. The questionnaire was administered to the genitors of the study group from March 2012 to September 2014. The data collection from the control group occurred from May to September 2014.

Before using the questionnaires, formal consent was obtained from the executive board of both hospitals; Ethics in Research Committee approval (processes 003/2012 and 560,994/2014) were received and all participants signed the Informed Consent Term.

Study variables
Presence of Cleft lip and palate was the dependent variable. Independent variables related to the child: gender; age; race/color of skin (according to the classification of the Brazilian Institute of Geography and Statistics) [22] and order of pregnancy. Independent variables related to parents: age and education level. Independent variables related to maternal exposure in the first trimester of pregnancy: occurrence of diabetes, hypertension, infection, obesity; use of medication (vitamin supplement, folic acid, analgesic, antibiotic, anti-inflammatory, corticosteroids, anticonvulsants and benzodiazepines); alcohol consumption, (active/passive) smoking, illicit drug use and exposure to ionizing radiation.

Processing and data analysis
Data processing was carried out in an Excel spreadsheet and the statistical analyzes were performed with the
Tables presenting absolute and relative frequencies were used for the descriptive data analysis. In the inferential analysis, measures of association between dependent and independent variables were determined using Chi-square and Fisher's exact tests and Likelihood Ratio with a 0.05 significance level.

The crude odds ratio (OR) with their respective intervals of confidence of 95% (CI 95%) and the association between variables were also obtained, and those with $p < 0.20$ were considered for the construction of the multivariate logistic regression model, remaining in the final model the variables with significance level inferior than 0.05 ($p < 0.05$).

Results
Regarding the socio-demographic data of the study population ($n = 450$), the predominance of male children ($64.67\%$, $n = 292$), of white race/color of skin ($43.56\%$, $n = 196$) was observed. $63.78\%$ ($n = 287$) of the mothers aged from 20 to 34 years and $82.89\%$ ($n = 373$) of the fathers aged from 20 to 39 years. The level of education of 10 to 12 years of study prevailed in $63.11\%$ ($n = 284$) of mothers and $50.67\%$ ($n = 228$) of the fathers.

As for the data related to pregnancy, prenatal, occurrence of diseases, use of medication and social habits in the first trimester of pregnancy, it turns out that $97.78\%$ ($n = 440$) of women received prenatal care and $40.22\%$ ($n = 181$) were in first pregnancy. Only $2.44\%$ ($n = 11$) of mothers had diabetes, $7.33\%$ ($n = 33$) hypertension, $33.55\%$ ($n = 151$) infection, predominantly urinary and vaginal; $41.78\%$ ($n = 188$) psychological stress and $8.67\%$ ($n = 39$) were overweight at the beginning of pregnancy. Among medications use, the most commonly ones are the folic acid ($71.33\%$, $n = 321$), vitamins ($50.67\%$, $n = 228$), analgesics ($46.44\%$, $n = 209$), antibiotics ($25.33\%$, $n = 114$) and anti-inflammatory ($4.67\%$, $n = 21$). Seventy-four women ($16.22\%$) reported alcohol consumption, $1.56\%$ ($n = 7$) illicit drug use, $21.78\%$ ($n = 98$) active or passive smoking and $2.44\%$ ($n = 11$) contact with ionizing radiation.

Tables 1 and 2 show the bivariate analysis of data, observing association ($p < 0.05$) between the following variables and the occurrence of oral clefts: Native-American race/color of skin ($p < 0.001RV$), father education level ≤9 years ($p = 0.007$), exposure to ionizing radiation ($= 0.008EF$), passive smoking ($p = 0.010$) and obesity ($p = 0.013$). Being of black race/color of skin ($p = 0.006$) and the use of analgesic ($p < 0.001$) and folic acid ($p < 0.001$) were associated with lower risk of occurrence of oral clefts. No association was found with the other studied variables.

The factors associated with the outcome after analysis on the logistic regression multivariate model are presented in Table 3. The variables that remained as a risk factor for the occurrence of oral clefts by maternal exposure in the first trimester of pregnancy were: obesity ($p = 0.001$), passive smoking ($p = 0.010$) and exposure to ionizing radiation ($p = 0.015$). The use of antibiotics was associated in the multivariate analysis ($p = 0.002$). The analgesic use was confirmed as a preventive factor ($p < 0.001$) as well as folic acid ($p < 0.001$).

Discussion
Oral clefts are the oral malformations of higher incidence of in the world population [1-2], however the factors associated with its pathogenesis are still not completely defined [2-3]. So it is vital that new research be conducted with the purpose of helping to unravel the etiology of this important public health issue [4].

Once gene therapy is still not available for the prevention of oral clefts [1, 4] this study adhered to the investigation of maternal exposure to risk factors related to its occurrence within the first trimester of pregnancy. The identification of such factors would enable the establishment of preventive measures to prevent or control exposure by pregnant women, especially during the first trimester of pregnancy.

The decision of pairing the mothers of the case and control groups considering the gender of their children but not their age occurred in order to reduce the recall bias of the mothers from the control group.

In births, where parents are faced with a very different child rather the idealized one, it naturally begins an internal process of finding a cause to explain the problem to minimize the feeling of guilt, so the pregnancy memories become more vivid. Baby without malformations and close to the idealized one become more difficult to be remembered over time [23]. Thus, the collection of data from the control group was conducted
Previous studies have observed a greater association of pregnancy obesity and ten birth defects, including isolated cleft palate [16]. A study in Texas-EUA [17] found substantially increased risk of birth defects among obese mothers (BMI≥30), including cleft lip with or without cleft palate. Stott-Miller et al. [28] also observed increased risk of isolated orofacial clefts among children of obese women.

Table 1: Association between sociodemographic variables and the occurrence of oral clefts.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Categories</th>
<th>Cases</th>
<th>Controls</th>
<th>OR (95%CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Child sex</td>
<td>Male</td>
<td>97 33.33 194 66.67 1.00 (0.66 ; 1.51)</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>53 33.33 106 66.67 1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Child race/color</td>
<td>Native-American</td>
<td>5 100 0 0.00 -</td>
<td>-</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Black</td>
<td>24 20.17 95 79.83 0.48 (0.28 ; 0.81)</td>
<td>0.006</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Brown</td>
<td>53 40.77 77 59.23 1.30 (0.82 ; 2.05)</td>
<td>0.266</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>≤19 years</td>
<td>14 41.18 20 58.82 1.41 (0.69 ; 2.88)</td>
<td>0.350</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥20 years</td>
<td>12 27.91 31 72.09 0.78 (0.39 ; 1.57)</td>
<td>0.480</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Father’s age at pregnancy</td>
<td>20 a 39 years</td>
<td>124 33.24 249 66.76 1.00</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥40 years</td>
<td>12 27.91 31 72.09 0.78 (0.39 ; 1.57)</td>
<td>0.480</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother’s age at pregnancy</td>
<td>20 a 34 years</td>
<td>94 32.75 193 67.25 1.00</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥35 years</td>
<td>10 31.25 22 68.75 0.93 (0.42 ; 2.05)</td>
<td>0.863</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Father’s education level</td>
<td>9 years</td>
<td>58 42.34 79 57.66 1.84 (1.18 ; 2.87)</td>
<td>0.007</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt; 12 years</td>
<td>19 39.58 29 60.42 1.64 (0.86 ; 3.14)</td>
<td>0.130</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Not informed</td>
<td>8 21.62 29 78.38 0.69 (0.30 ; 1.59)</td>
<td>0.384</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother’s education level</td>
<td>9 years</td>
<td>45 38.79 71 61.21 1.41 (0.90 ; 2.21)</td>
<td>0.133</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt; 12 years</td>
<td>17 34.00 33 66.00 1.15 (0.61 ; 2.17)</td>
<td>0.672</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

OR: Odds Ratio; CI: confidence interval; p-value: Chi-square test.

with the mothers still hospitalized in the postpartum.

Among the sociodemographic variables analyzed in the present study, the Native-American race/color was associated with the occurrence of oral clefts (< 0.001RV). Although similar results have been reported in studies conducted in other countries [13-14], it is possible that the incidence of congenital anomalies among Native-American be often underestimated due to the existence even nowadays of infanticide of malformed children [24]. In the present case, the association found may be related to the occurrence of consanguineous marriages, common in native communities [24-25]. However, the presence of Native-American individuals only in the case group should be considered with caution because it may represent an artificial result, not necessarily associated with a higher incidence of oral clefts in this population. While oral cleft natives search for treatment at the state capital referral service, native pregnant women hardly move to the capital to have their children, so that childbirth usually occurring in the own community [24-25] or in health institutions closer to the villages. Thus, the chance of Native-American being included in the control group is naturally less likely than in the case group.

Being of black race/color was considered in this study as a preventive factor to the oral clefts occurrence. Previous studies have observed a greater association between oral clefts and whites followed by brown [14], or brown followed by white [15], and a lower prevalence of all types of oral clefts among blacks compared to whites and Asians [13-14, 26]. While studies have shown a significant relationship between the educational level of the mother and the risk of their occurrence [11, 15]; others showed no interference between schooling and the occurrence of oral clefts [27-28].

This study showed an association between low paternal education and the occurrence of oral cleft (p = 0.007), unlike other studies that only analyzed the maternal level of education [11, 27-28]. Whereas education is directly related to income [22], low schooling of the father may have hindered the pregnant women access to adequate nutrition, contributing to the occurrence of congenital malformation.

Obesity has been identified as a risk factor for fetal malformations, such as neural tube defects, heart defects and orofacial clefts [16].
In this study the occurrence of cleft lip and palate was associated to obesity ($p = 0.013$), but not to diabetes ($p = 0.518$) and hypertension ($p = 0.055$), however the p-value was close to the statistical significance threshold for the association of cleft lip and palate and the mother’s hypertension. The results showed that the incidence of births of children with oral clefts was strongly associated with non-folic acid supplementation by the mother.
in the first trimester of pregnancy \( (p<0.001) \), representing an increase of 2.94 to 3.17 in the chance of oral cleft occurrence \( (3.94;1.41;17.17-1) \). The preventive effect of folic acid and vitamin supplements in the occurrence of cleft lip with or without cleft palate is a consensus \([11-12,29]\).

In this study, the use of analgesics showed strong preventive association in the occurrence of oral cleft \( (p<0.001) \). That can be attributed to the relaxing effect of the cessation of pain, as described in a previous study \([30]\), which suggests that the physical and/or emotional stress may be implicated in the occurrence of oral clefts. The longer duration high tension can cause oxidative damage at cellular level by disruption of the hypothalamic-pituitary-adrenal axis leading to high levels of cortisol and cytokine production. This hormonal change leads to a decrease of the blood supply in the muscles, leading to decreased blood flow to the placenta causing a nutritional deficiency that can lead to genetic abnormalities in the fetus \([30]\). Studies have shown an association between the use of antibiotics, such as tetracycline, sulfamethoxa zole, trimethoprim, pivmecillinam \([19]\) and amoxicillin \([9]\) in early pregnancy and the risk of isolated orofacial clefts. Rocha et al. \([8]\) found no statistical significance between the teratogenic risk for the use of antibiotics during pregnancy and the presence of fetal malformations. In the multivariate analysis results of this study, the use of antibiotics in early pregnancy was associated with the risk of oral clefts \( (p=0.002) \) and deserves further investigation concerning the type and the prescribed dosage of antibiotic, given that the study was limited to investigating only the use or not use of the drug.

Smoking during pregnancy has often been associated with risk of oral clefts \([6,9]\) regardless of race/color \([5]\), because of reduced blood exchange between mother and fetus and fetal folate levels \([9]\). In this study passive smoking of pregnant women was associated with the occurrence of oral clefts in their children \( (p=0.010) \), observing even greater association in the group of children whose father smoked more than 21 cigarettes per day during the first trimester of pregnancy \( (p<0.001) \).

The low number of pregnant smokers in this study may be related to the disclosure to the general population and during the prenatal consultations that smoking can induce the occurrence of birth defects. The association found between oral clefts and passive smoking, that is, the smoking habit of the partner, indicates that conducts aimed to reduce this habit from the partner and mother’s close people during pregnancy need to be disclosed as an important preventive measure for birth defects.

Another possibility is that the paternal smoking may exert some influence even before the pregnancy, interfering in the genesis of the male gamete that would end up generating a child with oral cleft \([7]\). This possibility to be confirmed requires further studies using different methodologies.

Although it was shown that the fetus is more susceptible to radiation between the second and fifteenth weeks of intrauterine life in a research on the risk of exposure to ionizing radiation resulting from medical procedures, Patel et al. \([20]\) concluded that there is no acknowledged risk to the development of congenital malformation to fetuses exposed to ionizing radiation at levels typically used for diagnostic imaging. Rakotaroison et al. \([21]\) pointed out the high doses of ionizing radiation from former uranium mines as a possible explanation for the high cleft prevalence in the Vakinankaratra region in Madagascar. In this study exposure to ionizing radiation was associated with the presence of oral clefts \( (p=0.015\text{EF}) \). The result, howev-

<table>
<thead>
<tr>
<th>Variables/Categories</th>
<th>AOR</th>
<th>CI (95%)</th>
<th>( p ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obesity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overweight grades 1 and 2</td>
<td>3.41</td>
<td>(1.61;7.26)</td>
<td>0.001</td>
</tr>
<tr>
<td>No</td>
<td>1.00</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Use of folic acid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1.00</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>4.17</td>
<td>(2.60;6.68)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Use of analgesics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0.35</td>
<td>(0.22;0.55)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No</td>
<td>1.00</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Use of antibiotics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2.24</td>
<td>(1.35;3.72)</td>
<td>0.002</td>
</tr>
<tr>
<td>No</td>
<td>1.00</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active</td>
<td>1.53</td>
<td>(0.61;3.87)</td>
<td>0.366</td>
</tr>
<tr>
<td>Passive</td>
<td>2.18</td>
<td>(1.23;3.93)</td>
<td>0.010</td>
</tr>
<tr>
<td>No</td>
<td>1.00</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>X-ray</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>6.95</td>
<td>(1.46;33.15)</td>
<td>0.015</td>
</tr>
<tr>
<td>No</td>
<td>1.00</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

AOR: adjusted odds ratio. CI (95%): confidence interval of 95%. P values highlighted in bold are statistically significant \( (p<0.05) \). Logarithm of likelihood value of the model = -246.9055 and \( p \) value of the model <0.001.
er, needs to be further explored, as the questionnaire limited the response to yes or no for questions whether mothers have been subjected to ionizing radiation in the first trimester of pregnancy or not. Thus, information on the frequency, body site and ionizing radiation levels to which they were submitted were not investigated, indicating a need for future studies.

The findings of this study confirm the relationship between the occurrence of cleft lip and palate and behavioral maternal gestational conditions therefore likely to be prevented. Thus, the need for the monitoring of pregnant women is reiterated, especially in the first trimester of pregnancy, in order to limit or control their exposure to factors that were associated with its occurrence.

Conclusion
Considering the methodology used in this study, it can be concluded that obesity, passive smoking, exposure to ionizing radiation and antibiotic use in the first trimester of pregnancy are associated with the occurrence of cleft lip and palate. The use of folic acid and analgesics and being of black race/color presented preventive effect for its occurrence. Thus, monitoring and careful controlling the identified risk factors in pregnant women, especially in the first trimester of pregnancy, is essential.

Future studies are needed to clarify the relationship between risk factors and protective factors identified in this study and the occurrence of cleft lip and palate in the population.

Acknowledgment
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Conflict of Interest
The authors declare that they have no conflict of interests.

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[11] Figueiredo RF, Figueiredo N, Feguri A, Bieski I, Mello...


