Chemical exposure during pregnancy and oral clefts in newborns

Exposição a agentes químicos na gravidez e fendas lábio-palatinas no recém-nascido

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Abstract *This article presents a literature review on the risk factors for oral clefts (lip and/or palate), emphasizing discussion of maternal exposure to endocrine disruptors. Several studies have identified the risk of cigarette smoking and alcohol consumption, use of anticonvulsivant drugs, and exposure to organic solvents. A protective effect has been shown for supplementation with folic acid. As with other chemicals, the risk associated with exposure to sex hormones is still obscure, although some authors describe a moderate risk level. New studies addressing this hypothesis need to be conducted, while the population exposed to these endocrine disrupters is increasing.*
Key words *Cleft Palate; Cleft Lip; Hormones; Risk Factors*

Resumo *O presente artigo apresenta uma revisão bibliográfica sobre os fatores de risco para a ocorrência de fendas lábio-palatinas descritos na literatura, destacando a discussão sobre a exposição hormonal materna durante a gravidez. Os trabalhos analisados apontam como fatores de risco o tabagismo e a ingestão de álcool, uso de anticonvulsivantes e exposições a solventes orgânicos, e como fator de proteção, a administração de ácido fólico. O risco associado à exposição hormonal, bem como a outros fatores, ainda é obscuro, embora alguns autores descrevam moderadas magnitudes de risco. Novos estudos, especificamente elaborados para testar esta hipótese, devem ser realizados à medida em que aumenta a população exposta a drogas de ação endócrina.*
Palavras-chave *Fissura Palatina; Lábio Fissurado; Hormônios; Fatores de Risco*

Introduction

Neural tube defects and oral clefts are among the most common congenital malformations in humans. Although they are etiologically distinct, both involve genetic and environmental components in their development (Finnell et al., 1998). Although facial clefts have been reported for centuries, their etiology has not been clearly established (Gordon & Shy, 1981).

Different factors have been described to explain such clefts, including insufficient embryological development, partial failure in the fusion of the medial nasal processes, and developmental abnormalities. The genetic component is considered key to the appearance of orofacial clefts, explaining 25-30% of the observed cases (Tolarová & Cervenka, 1998). As for environmental exposure, potential teratogenic factors can be classified into five categories: infectious agents (cytomegalovirus associated with cerebral atrophy and cerebral palsy; teratogenic action of the rubella virus resulting in the congenital rubella syndrome, for example), ionizing radiation (with evidence of association with microcephaly, mental retardation, and skeletal malformation), licit or illicit drugs (fetal alcohol syndrome; neurobehavioral disorders and microcephaly associated with cocaine; use of methotrexate resulting in multiple malformations of the skull, face, limbs, and spinal column), hormones (feminization of male fetuses and hypospadias), and nutritional deficiencies (neural tube defects related to folic acid deficiency; malformations resulting from hypervitaminosis A), as described by Ten Cate (1988) and Moore & Persuad (1995).

This article provides a state-of-the-art literature review on the association between environmental and occupational risk factors in the development of orofacial clefts, highlighting hormone exposure in the context of their causality.

Search methodology

The MEDLINE bibliographic database was consulted for the period from 1970 to 2000, using the following key words: oral clefts, risk factors, oral contraceptives, sex hormones, endocrine disruptors. Observational studies were selected that clearly identified the respective control groups, as well as an evaluation of outcomes and exposures with equivalent procedures for comparing the two groups.

Risk factors

Smoking

The association between maternal smoking during pregnancy and the development of orofacial clefts has been inconsistent across different studies. There is evidence relating maternal smoking to low birth weight, premature birth, and perinatal mortality. However, the association with malformations is controversial, and although most studies indicate a positive association, its statistical significance has not always been demonstrated (Källén, 1997).

Werler et al. (1990) reviewed various studies involving mothers of children with congenital malformations, seeking to associate environmental exposures to the prenatal events. The various stratifications for smoking according to the number of cigarettes smoked per day generated risk estimates (odds ratios – OR) close to one, while multivariate analysis controlling for potential confounders failed to alter such estimates. Thus, they concluded that smoking during pregnancy appears not to significantly increase the risk of developing cleft lip with or without cleft palate (CL/P). Although no statistical association was found, the authors did not rule out the teratogenic effect of smoking, while suggesting that it was probably small.

A case-control study by Källén (1997) showed an association between smoking and cleft palate (CP) of 1.29 (95% CI: 1.08-1.54), greater than that observed for CL/P, on the order of 1.16 (95% CI: 1.02-1.32). Wyszynski & Beaty (1996) collected studies conducted from a period of three decades (from 1966 to 1996) on the association between smoking and this group of malformations, analyzing a total of 10 studies. The standardized OR for these studies was 1.29 (95% CI: 1.18-1.42) for cases of CL/P and 1.32 (95% CI: 1.09-1.60) for CP, with the exposure concentrated in the first trimester of pregnancy and indication of a dose-response effect. Attributable risk for smoking was 11% for CL/P and 12% for CP. According to the authors, the principal confounders for this association were diet and maternal age.

Lieff et al. (1999) analyzed the effects of smoking on malformations, observing only a moderate rise in the risk of cleft lip with cleft palate (CLP) associated with other malformations, attributing the association of smoking to this other form of congenital alteration.

Lorente et al. (2000) obtained a risk estimate of 1.79 (95% CI: 1.07-3.04) for CL/P. They detected an upward risk gradient with the increase in the magnitude of exposure. However,

this association disappeared when the statistical model was adjusted for alcohol consumption.

In short, the above studies generally showed a weak association between smoking and the occurrence of this group of malformations, sometimes failing to reach statistical significance.

Alcohol

Among the abnormalities characterizing fetal alcohol syndrome are alterations of the craniofacial structures. The fact that the latter derive embryologically from neural crest cells leads to the hypothesis that alcohol exerts a teratogenic effect on normal development and migration of these cells. Although experimental models have shown the association between alcohol and birth defects like orofacial clefts, these findings are obscure in humans. Munger et al. (1996) studied the influence of alcohol intake and the occurrence of malformations, conducting a population-based case-control investigation. Adjusting the model obtained by variables like family income, maternal education, smoking, vitamins, child's age, and year of birth, they observed an increasing risk gradient with increased consumption of alcohol doses/month for cases of CL/P, where for the highest doses consumed the OR was statistically significant (4.0; 95% CI: 1.1-15.1). In light of these findings, the authors conclude that there may in fact be a real association between alcohol intake during pregnancy and CLP.

In a multicenter study conducted in Europe with 161 cases of patients with oral clefts, Lorente et al. (2000) detected an increase in the magnitude of risk associated with alcohol consumption and the occurrence of cleft palate (OR: 2.28; 95% CI: 1.02-5.09), highlighting the relevance of these exposures in this group of malformations. However, a dose-effect response was not shown.

In short, there are few studies that specifically evaluate the potential association between oral clefts and maternal alcohol consumption. The studies described above indicate a greater association than that for smoking, although no dose-effect response is evident (Table 1).

Occupational exposures

The importance of studying the harmful effects of occupational exposures is based on the fact that they can affect both parental germ cells before conception and fetal somatic cells after conception. The two mechanism can induce cell death or dysfunction, resulting in malformation (Shaw & Gold, 1988).

Maternal occupations related to transportation and communications were significantly associated with oral clefts (OR: 1.94; $p < 0.05$) in a study by Hemminki et al. (1980), who analyzed a potential association between parents' occupation and these three groups of malformations (central nervous system and muscular-skeletal) in their offspring. Referring to solvent use, Holmberg et al. (1982) indicated that mothers of cases were more exposed to this heterogeneous group of substances than mothers of controls, especially to aliphatic and aromatic hydrocarbon and their mixtures.

A study by Cordier et al. (1992) focused on all offspring with major birth defects detected in the prenatal or perinatal period (from the 7th gestational month to the 7th day of life). The principal exposure analyzed was to solvents, and the principal group of workers were those involved in hospital activities. Exposure to solvents and the occurrence of clefts generated an OR of 7.9 (90% CI: 1.8-44.9). Although they were not significant, increases in risk were also observed for exposure to such products as detergents and disinfectants. Due to the small number of observations, the estimates are not very precise. In relation to this same group of substances, Laumon et al. (1996) studied exposure to organic solvents during pregnancy, whose association with orofacial clefts proved inconsistent. Mothers were asked about their childbearing history and exposures during the first two months of pregnancy. The association between typical orofacial clefts and solvent use was reflected by an odds ratio of 1.62 (95% CI: 1.04-2.52). However, when analyzing by isolated sub-groups of solvents, only exposure to aliphatic halogen solvents was statistically significant (OR: 4.40; 95% CI: 1.41-16.15), a result that was not altered when stratified by other variables.

Cordier et al. (1997) observed the effects of occupational exposures in women before and during pregnancy on the occurrence of malformations. Exposure to chemical compounds was evaluated one month before pregnancy and during the first trimester. In this study, mothers of controls resided more frequently in urban areas and worked as liberal or administrative professionals. A strong association was detected between orofacial clefts and exposure to solvents (OR: 7.9; 95% CI: 1.88-44.9), used especially in dry-cleaning activities. Exposure to glycol ether during the first trimester of pregnancy was associated with the occurrence of clefts, with an OR of 1.97 (95% CI: 1.2-3.25),

Table 1

Epidemiological studies on the association between orofacial clefts and smoking/alcohol consumption.

Author (association studied)	Country (period)	Methodology	Results	Comments
Werler et al., 1990 (smoking and orofacial clefts)	United States/ Canada (1983-1987)	Case-control	The estimated risks do not suggest an association. OR for CL/P only 1.2 (0.9-1.6); 1.4 (1.0-2.1) and 0.7 (0.3-1.6) and for CP 1.0 (0.7-1.5); 0.9 (0.5-1.5); 0.8 (0.3-2.2), with smoking of 1 to 14; 15 to 24 and > 25 cigarettes per day.	Hospital controls were used, excluding those with birth defects potentially associated with smoking.
Källén, 1997 (smoking and orofacial clefts)	Sweden (1983-1992)	Case-control	OR for CL/P: 1.16 (1.02-1.32). OR for CP: 1.32 (1.10-1.62).	The observed dose-response effect for the CL/P vs. smoking association was directly related to the cleft palate group.
Munger et al., 1996 (alcohol consumption and orofacial clefts)	United States (1987-1991)	Population-based case-control	For CL/P there was a significant increase in the magnitude of risk directly related to increased maternal exposure during pregnancy: 1.5 (0.9-2.4); 3.5 (0.3-15.4); 4.0 (1.1-15.1) in the adjusted analysis, varying from 1 to 3 drinks/month to > 10 drinks/month.	Cases included live births, stillbirths, and abortions. Among controls, the refusal rate was 45.4%, with the inclusion of only live births.
Wyszynski et al., 1997 (smoking and orofacial clefts)		Meta-analysis	OR for CL/P: 1.29 (1.18-1.42). OR for CP: 1.32 (1.10-1.62).	Of the 11 studies analyzed, 9 were case-control type. Of the 8 studies that allowed for analyses of increased risk in relation to exposure, 5 demonstrated a dose-response effect with a χ^2 showing a significant trend.
Lieff et al., 1999 (smoking and orofacial clefts)	United States (1976-1992)	Case-control	An association was observed between smoking and CL/P associated with additional malformations, including a dose-response effect when the number of cigarettes per day was analyzed.	Possible confounders and effect modifiers were analyzed, including family history of clefts and folic acid in diet or supplements. The analysis was divided into two periods (1976-1982 and 1988-1992), since beginning in 1988 questions related to smoking and alcohol consumption were explored in greater depth. Some inconsistencies in the data can be attributed to this variation in evaluating exposure.

while for cases of CP it was 1.68 (95% CI: 0.7-3.76) and for CL/P 2.03 (95% CI: 1.11-3.73). Such associations were not altered when stratified by maternal age, area of residence, and socioeconomic status. Glycol ether is associated with increased incidence of spontaneous abortion and decreased fertility and is a common ingredient in products widely used in domestic cleaning activities (Correa et al., 1996).

Bianchi et al. (1997) comment that the literature suggests an association between oral clefts and exposure among health workers like physicians and maintenance/repair personnel. In the health field, some associations were shown, albeit inconclusive, with exposure to anesthetic gas and contact with cytostatic drugs (without the definition of a specific biological model). However, a strong association was mentioned between cleft lip and maternal occupation in the leather and shoemaking industry (OR: 3.9; 99% CI: 1.5-9,8) increasing considerably for cleft palate only (OR: 5.4; 99% CI: 1.8-13.4). Moderate associations were also found for hairdressers (OR: 2.2) and dry-cleaning workers (OR: 1.9). Cleft palate showed a particularly high and statistically significant association with the leather industry (OR: 5.0; 95% CI: 1.2-14.6). The leather industry involves intense exposure to solvents, especially aliphatic hydrocarbons, chlorinated hydrocarbons, and other aromatic solvents, although it is generally not possible to precisely measure the in-

tensity, degree, and duration of the worker's overall exposure. Despite these findings, the study's power was limited, and it was not possible to rule out biases ocurence.

A case-control study in Spain by García & Fletcher (1998) sought to evaluate the association reported in the literature between occupational exposures in the leather industry and various groups of birth defects by applying a questionnaire. Orofacial clefts were the birth defects with the strongest association to this exposure, particularly in assembly lines working with leather (OR: 6.18; 95% CI: 1.48-25.69), although only 18 cases were analyzed.

Thus, different studies conducted in different population groups and distinct time periods show the association between oral clefts and exposure to a heterogeneous group of solvents (Table 2). The action of these chemical agents has already been described in the literature on risk factors for other diseases, like some neoplastic diseases, including non-Hodgkin lymphomas, leukemias, and pancreatic tumors (Ojajarvi et al., 2000; Rego, 1998). These findings tend to ensure the biological plausibility of their possible involvement in disturbing cell division mechanisms.

Environmental exposures

Common sense associates the potential impact on reproductive health of exposure to contaminants in hazardous waste sites, where the most commonly found products are residues of solvents, pesticides, and metals.

Since the 1940s there has been a dramatic rise in the use of agricultural inputs, including herbicides, insecticides, and fungicides. Such chemical substances are found throughout the environment and are manufactured specifically to be toxic to certain organisms, while some are known or suspected to be teratogenic, mutagenic, or carcinogenic in animals. Even so, relatively little attention has been paid to hundreds of chemical formulations and their effects on the health of populations. In light of these arguments, Gordon & Shy (1981) raised the hypothesis that intrauterine exposure to agricultural chemicals in farming areas during periods of peak use of pesticides and herbicides, especially in the first trimester, could be associated with increased risk of birth defects. This ecological study displayed the peculiar limitations of generalizing data, but shows independent effects of exposure to these products for the occurrence of clefts. The study should not be considered definitive, but it did raise a hypothesis for further investigation.

Known sources of pesticide exposure include maternal farm work, maternal residence in farming areas, and maternal gardening. Shaw et al. (1988) studied a total of 215 cases of orofacial clefts among both live births and stillbirths with more than 20 weeks' gestation. With regard to gardening exposure, professional application of pesticides produced an OR of 3.8 (95% CI: 1.5-9.7) for the occurrence of CLP associated with other malformations. For CLP only the OR was 1.5 (95% CI: 0.5-4.1) for activities related to orange growing. Although the results show a moderately increased risk in some cases, they do not present a clear pattern of association with exposure to specific pesticides.

Croen et al. (1997) analyzed geographic location (risk areas) and possible association with environmental factors (chemical contamination) and congenital disorders. Areas were classified according to mean environmental contamination (air, biota, oil, surface water, water springs, and specific chemical contaminants). A relative risk of over 2.0 was found for cases residing in environmental risk areas, suggesting that despite possible methodological problems, there was a greater probability of developing malformations in these areas, with a special focus on chemical substances such as cyanides (OR: 1.3; 95% CI: 0.3-5.6) and inorganic compounds (OR: 1.3; 95% CI: 0.5-3.4). The observed associations may have been influenced by other, uncontrolled chemical sources like occupation, industrial emissions, pesticides, and water contamination not associated with the target waste sites.

Thus, despite difficulty in measuring toxic environmental agents, the studies suggest that environmental contamination is associated with the occurrence of malformations.

Use of medication during pregnancy and implications

The international literature includes several studies seeking to identify the teratogenic effect of various medications, including the classic thalidomide studies in the 1960s (McBride, 1961). With regard to orofacial clefts (Table 3), the most extensively identified association was between the use of anticonvulsivant drugs and cleft lip with or without cleft palate, especially with the use of diphenylhydantoin, phenobarbital, and multi-drug therapy (Castilla et al., 1996). Various authors observed a two-fold risk of birth defects among children of epileptic mothers undergoing this form of prolonged treatment during pregnancy (Hill et al., 1988; Wyszynski et al., 1996).

Table 2

Epidemiological studies on the association between orofacial clefts and environmental and occupational factors.

Author (association studied)	Country (period)	Methodology	Results	Comments
Hemminki et al., 1980 (occupational factors and malformations)	Finland (1967-1977)	Case-control	OR = 1.94 ($p < 0.05$) for mothers working in transportation and communications sectors.	Except for the age and place-of-birth variables, there is no reference to controlling of potential confounders. The heterogeneous set of orofacial clefts was studied as a whole.
Gordon & Shy, 1981 (environmental factors – pesticides – and orofacial clefts)	United States (1974-1975)	"Case-control" using ecological data to analyze exposures	The study did not detect differences in exposure between cases and controls. Birth during winter months appeared to involve greater risk, regardless of sex.	The case control ratio was 1:5. Exposure to pesticides was estimated by the quotient between the number of acres of a crop using pesticides divided by the total acres available for farming activities. The exposure estimate was not highly precise, using analysis of isolated pesticides per area, whereas pesticide use is generally combined.
Holmberg et al., 1982 (occupational factors and clefts)	Finland (1977-1980)	Population-based case-control	Mothers of cases tended to be more exposed to solvents than controls ($\chi^2 = 4.5$, $p < 0.05$).	The study evaluated the set of cases with and without other malformations, which can affect the observed associations.
Shaw et al., 1988 (environmental factors and malformations)	United States (1987-1988)	Case-control	OR suggestive of association between pesticides in gardening and CL/P only.	Analysis differed between clefts only and those associated with other birth defects.
Cordier et al., 1992 (environmental factors – solvents – and malformations)	France (1984-1987)	Case-control	OR = 7.9 (90% CI: 1.8-44.9) for orofacial clefts and exposure to solvents.	Cases included life births, stillbirths, and fetuses of any gestational age. Estimates not highly precise (reduced number of exposed fetuses).
Bianchi et al., 1997 (occupational factors and malformations)	Italy (1982-1989)	Case-control	OR = 3.9 (99% CI: 1.5-9.8) for manufacturing activities involving leather. Increased risk observed for CP only (5.4, with 95% CI: 1.8-13.4).	Exploratory study with weak power due to the small number of cases per occupation analyzed. Unmatched controls.
Cordier et al., 1997 (occupational factors and malformations)	EUROCAT (1989-1992)	Multi-center case-control study	OR = 1.97 (1.20-3.25) for maternal exposure to glycol ether during the first trimester of pregnancy.	Despite the small number of exposed individuals, increased risk was observed according to level of exposure.
Croen et al., 1997 (environmental factors – environmental risk areas – and malformation)	United States (1989-1991)	2 population-based case-control studies	OR > 2 only for cases of CL/P associated with other malformations in children born in chemically contaminated areas, especially with cyanides and inorganic compounds.	Combined analysis of different types of clefts. Wide confidence intervals (small number of exposed).
García & Fletcher, 1998 (occupational factors and malformations)	Spain (1993-1994)	Case-control	OR = 6.18 (1.48-25.69) for exposure in leather-handling industry.	The authors suggest that there is greater exposure to organic solvents in leather handling.

EUROCAT = European Registration of Congenital Anomalies and Twins.

Table 3

Epidemiological studies on association between orofacial clefts and use of medicines.

Author (association studied)	Country (period)	Methodology	Results	Comments
Rosenberg et al., 1983 (diazepam and orofacial clefts)	United States/ Canada (1976-82)	Case-control	OR = 0.8 (0.4-1.7) for CL/P. OR = 0.8 (0.2-2.5) for CP.	Control of results according to various potential confounders like life style, medical history, family history of malformation.
Hill et al., 1988 (medicines and malformation)	England (1983-1984)	Case-control	Use in 3 months prior to pregnancy: steroid hormones [OR = 1.5 (1.1-2.1)]; sulfas [OR = 2.4 (1.1-5.3)]; anti-convulsivants [OR = 5.3 (1.8-16.0)] Use in first trimester of pregnancy: anti-convulsivants [OR = 8.5 (2.5-28.8)].	The study analyzed the use of medicines during the three months prior to pregnancy and the first trimester. Exposure data were obtained by reviewing patients' clinical records.
Bracken, 1990 (oral hormones and malformations)		Meta-analysis	Overall OR = 0.99 (0.83-1.19) for association between oral contraceptives and malformations.	Twelve studies were selected. No specific data are presented for CL/P. The analysis for cardiac defects and limb reduction also suggests a lack of association.
Loffredo et al., 1994 (medicines and orofacial clefts)	Brazil	Case-control	Estimated risk for use of anti-inflammatory drugs and CL/P = 2.59 (CI = 1.35-4.98).	Hospital controls from different Brazilian States, since cases were from reference hospitals (reduction of control selection bias).
Czeizel & Rockenbauer, 1997 (corticosteroids and malformations)	Hungary (1980-1994)	Case-control	Use of oral corticosteroids and CL/P: OR = 1.27 (0.82-1.96). Use of topical corticosteroids and CL/P: OR= 2.21 (1.11-4.39)	High percentage of refusal among controls (35%).
Dolovich et al., 1998 (benzodiazepines and orofacial clefts)		Meta-analysis	OR= 1.19 (0.34-4.15) in cohort studies. OR=1.79 (1.13-2.82) in case-control studies.	Included 23 studies, among cohorts and case-controls. Concomitant exposures to other medicines may have overestimated the risk of benzodiazepine use.
Martínez-Frías et al., 1998 (oral hormones and orofacial clefts)	ECEMC (1976-1995)	Hospital-based case-control	OR for oral contraceptives and clefts = 1.38 (1.04-1.84). OR for combined estrogens (excluding progestogens) = 2.54 (1.01-6.43).	Non-malformed controls used. Exposure refers to use in first trimester of pregnancy. However, results of logistic analysis for hormone use showed an OR of 1.27 (0.76-1.78), displaying a stronger association with maternal childbearing history and family history of malformation.
Rodríguez-Pinilla & Martínez-Frías, 1998 (corticosteroids and orofacial clefts)	ECEMC (1976-1995)	Hospital-based case-control	CL/P and corticosteroids during first trimester of pregnancy with estimated risk of 6.55 (1.44-29.76).	Estimates controlled for confounders like smoking, maternal hyperthermia, family history of malformation, treatment with anti-convulsivants, benzodiazepines, metronidazole, and sex hormones.
Carmichael & Shaw, 1999 (corticosteroids and orofacial clefts)	United States (1987-1988)	Case-control	OR= 4.3 (1.1-17.2) for CL/P. OR= 5.3 (1.1-26.2) for CP.	Cases represented by live births and fetuses. Analyses performed for use of corticosteroids ranging from last month prior to pregnancy to first trimester.

ECEMC = *Estudio Colaborativo Español de Malformaciones Congénitas*.

In the 1970s, Saxén (1974) reported increased occurrence of cleft palate among children of mothers using benzodiazepines, particularly diazepam. Likewise, Safra & Oakley (1975) reported an increased association between the diazepam group and oral clefts as compared to other malformations. Meanwhile, a case-control study by Rosenberg et al. (1983) failed to corroborate these previous findings. Their controls were children with malformations, with the purpose of reducing potential recall bias. Their results suggest that the use of diazepam during the first trimester of pregnancy did not increase the risk of oral clefts. The investigation estimated relative risks, controlling for potential confounders, on the order of 1.2 (95% CI: 0.5-3.2) for cleft lip with or without cleft palate and 2.0 for cleft palate only (95% CI: 0.6-6.5), neither statistically significant.

Hill et al. (1988) observed an increased use of medication ranging from pre-conception through the first trimester of pregnancy among mothers of children with orofacial clefts, although not statistically significant. The most frequent report was the use of anti-convulsive drugs during the pre-gestational period.

A study by Loffredo et al. (1994) in the State of São Paulo (Southeast Brazil) focused on the risk factors most heavily associated with the occurrence of cleft lip with or without cleft palate. The authors found an association between these and family history (especially those involving the palate, with a relative risk of 2.89), history of maternal epilepsy (RR: 2.39), and use of anti-inflammatory drugs in the first month of pregnancy (RR: 2.59).

Dolovich et al. (1998) reviewed the available literature since 1966, and among cohort studies fetal exposure to benzodiazepines was not associated with increased risk of malformations or oral clefts (OR: 1.19; 95% CI: 0.34-4.15). Analyzing case-control studies, despite the severity of malformations, a reduced but significant association was observed, on the order of 1.79 for cleft palate (95% CI: 1.13-2.82). In these studies, the magnitude of association was not altered by using healthy as compared to malformed children as controls, suggesting that recall bias did not have a strong effect on the determination of excess observable risk.

Czeizel & Rockenbauer (1997) conducted a case-control study to evaluate the teratogenic potential of corticosteroid use, based on the type of administration (isolated or combined), administration route, dose, and gestational period of use, controlling for possible confounding variables (use of other drugs, medical history, and maternal age). Studying the association with CLP, they observed an OR of 1.27 (95% CI: 0.82-1.96) for oral use and 2.21 (95% CI: 1.11-4.39) for topical administration.

Given the inconsistency of studies on the association between corticosteroids and orofacial clefts, Rodríguez-Pinilla & Martíniez-Frías (1998) evaluated their use during pregnancy and the occurrence of malformations. A case-control study considered the following potential confounding variables during pregnancy: smoking, hyperthermia, maternal use of anti-epileptic drugs, benzodiazepines, metronidazol, and sex hormones. Based on their findings for the occurrence of CLP (OR: 5.2; 95% CI: 1.53-17.06), the authors recommend caution in the use of corticosteroids during gestation.

Evidence of corticosteroid teratogenicity in humans is limited and has resulted in inconsistent recommendations, particularly during early pregnancy. Carmichael & Shaw (1999) conducted a case-control study derived from a population-based investigation identifying cases of cleft lip and palate, neural tube defects, and limb reduction. They observed increased risk of cleft lip with or without cleft palate (OR: 4.3; 95% CI: 1.1-17.2) and cleft palate only (OR: 5.3; 95% CI: 1.1-26.5) subsequent to corticosteroid use. However, multivariate analysis showed no evidence of the control of this exposure by other potential confounders.

There is evidence of reduced risk for the occurrence of a series of malformations (clefts, cardiac defects, limb reduction, urinary tract defects, and cerebral defects, among others) based on vitamin supplementation before pregnancy and during early pregnancy (Werler et al., 1990). Werler et al. (1990) studied cases of newborns (up to 5 weeks of age) and abortions, both with defined malformations, compared to two different control groups: one including children with other malformations and the other including children without malformations. Mothers were interviewed about vitamin use during the two months prior to the last menstrual period and over the course of the trimesters. When cases were compared to non-malformed controls, there was a significant reduction in the risk of cleft palate only with vitamin supplementation during the first month (OR: 0.5; 95% CI: 0.2-1.4). Although not significant, there was also a reduced risk of cleft lip with or without cleft palate with vitamin supplementation at any time in the first trimester or prior to the last menstrual period. Calculating the combination of ORs for the time intervals related to the target malformation, once again the use of multivitamin supplements (at least 2 water-soluble and 2 fat-soluble types)

appeared to be a statistically significant protective factor (OR: 0.4; 95% CI: 0.2-0.8). The results were not substantially modified when compared with malformed controls. In this study, timing appears to be important for studying clefts; consistent with the embryological phenomenon, the greatest protective action against cleft lip occurs during the periconceptional period and for cleft palate during the second lunar month of pregnancy. Hayes et al. (1996) studied the protective effect of periconceptional use of folic acid supplements cited in the literature. Analysis of the data produced an odds ratio of 1.1 (95% CI: 0.8-1.7) for orofacial clefts, while for cleft palate the risk was 0.9 (95% CI: 0.5-1.6) and for cleft lip with or without cleft palate the risk was 1.3 (95% CI: 0.8-2.1).

The literature thus provides evidence that vitamin supplementation containing folic acid during pre-conception may reduce the risk of such malformations. However, this phenomenon has not been observed in all populations, suggesting the existence of genetic regulation in this phenomenon (Finnell et al., 1998). Various lines of experimental research demonstrate the biological plausibility of the use of folic acid in reducing the occurrence of orofacial clefts. The first evidence is from animal studies with the reduction of these malformations in litters submitted to folic acid-rich diets (Jordan et al., 1977; Nelson et al., 1960, *apud* Finnell, 1998). The second relates to women with apoplectic disorders using anticonvulsivant medication, known antagonists of folic acid, who are at increased risk of bearing children with this birth defect (Dansky & Finnell, 1991; Shaw et al., 1995). Given the possibility that fetal deficiency in both the transport and metabolism of folic acid places the fetus at increased risk for this malformation, and that maternal folic acid supplementation is not effective in all individuals, it is important to identify potential candidate genes for regulating this process (Finnell et al., 1998).

Werler et al. (1999) highlight that it is not clear which combination of nutrients or which specific nutrient is related to risk reduction for other malformations, since many vitamin supplements include 7 out of 8 water-soluble vitamins and 3 fat-soluble vitamins, in addition to at least 4 minerals or trace elements.

Evidence of association between oral hormones and orofacial clefts

Due to the critical role of hormones in the differentiation of various tissues, embryological development is particularly vulnerable to fluctuations in the period of administration or intensity of exposure to chemical agents with hormonal or hormone-like activity (Barlow et al., 1999). In the 1960s, Peterson (1969) studied cases of live births and detected an association between oral contraceptive use during pregnancy and various malformations, with a relative risk of 1.91 (95% CI: 0.25-14.46).

Classical studies highlighted the association between diethylstilbestrol (DES), a synthetic non-steroid compound with estrogen action widely used in the 1970s, and the development of rare types of vaginal tumors in adolescents whose mothers had been exposed to DES during the respective pregnancies and more frequently with vaginal adenosis and cervical pseudopolyps (Herbst et al., 1971; Newbold, 1995; Senekjian et al., 1988). However, there are doubts concerning the teratogenic capacity of more typical forms of hormone use, like progesterone and contraceptives, specifically in relation to non-genital congenital lesions (Castilla, et al., 1996).

Nora et al. (1978) associated hormone therapy during pregnancy to treat threatened abortion with increased frequency of malformations. Their study showed a strong association (RR: 8.41; $p < 0.001$) with the group of abnormalities known as VACTERL (vertebral abnormalities, anal atresia, cardiac abnormalities, tracheoesophageal fistula and/or esophageal atresia, renal agenesis and dysplasia, and limb defects). Evidence of a strong association was obtained for cases of congenital cardiac malformation (RR: 5.38; $p < 0.01$). Increased frequency of neural tube defects combining both the use of oral contraceptives and other forms of hormone exposure (associated with treatment of threatened abortion, hormone failure, anti-cancer treatment, and pregnancy tests, among others) have been described (Greenberg et al., 1977; Janerich et al., 1980; Nora et al., 1978).

On the other hand, Shardein (1980) contends that there is a series of limitations to the studies published on the teratogenic effect of oral contraceptive (OC) use, and that there is no justification for the prevailing concept on the induction of non-genital congenital malformations by OC use. The same is not true for the case of central nervous system malformations. According to the author, the risk effects appear to be non-specific, and in the case of orofacial clefts the association is controversial.

In relation to neural tube defects, Kricker et al. (1986) observed an association of 30.2 in the presence of history of OC use, possibly related to a recall bias.

A meta-analysis by Bracken (1990) attempted to estimate the risk of congenital malformations related to early OC exposure during pregnancy. Again, there was a lack of association between OC use and birth defects (OR: 0.99 for all malformations, 1.06 for congenital cardiac defects, and 1.04 for limb defects). Another meta-analysis by Raman-Wilms et al. (1995) combined studies testing the association between OC use and congenital genital malformations. After finding an OR of 1.09 (95% CI: 0.90-1.32) for use of hormones in general and this group of malformations and more specifically an OR of 0.98 (95% CI: 0.24-3.94) for OC use, they concluded that there was no association.

Martínez-Frías et al. (1998) conducted a case-control study using healthy newborns matched for age and hospital of birth as controls. Exposure to sex hormones was defined as occurring in the first trimester of pregnancy. Studying the set of selected abnormalities, OC and combined estrogen use, excluding progestogens, proved to significantly increase the risk of birth defects. In the case of cleft lip with or without cleft palate, prenatal exposure to progestogens and other combinations excluding estrogens produced an OR of 5.11 (95% CI: 1.50-17.37). However, when this exposure was controlled for possible confounders (vaginal bleeding, malformations in first-degree relatives, history of abortion), the association decreased in magnitude (OR: 1.27; 95% CI: 0.76-2.13). Thus, this study's findings do not support the teratogenic effect of hormone exposure, indicating that if there is a risk for non-genital congenital defects it is small.

Hemminki et al. (1999) investigated the association between female sex hormones and the occurrence of estrogen-dependent tumors in mothers and children, as well as genital malformations. They did not show a risk effect for neoplasia (either in mothers or children), but they did identify a slight risk effect for the occurrence of malformations. This study thus supports the hypothesis that hormone therapy using estrogens or progestogens during the first trimester of pregnancy may be associated with these outcomes.

James (2000) comments that high hormone concentration at the time of conception may partially determine the offspring's sex. The author thus analyzed the sex ratio of healthy siblings of patients with CLP, noting a trend towards a higher sex ratio among siblings of individuals with CLP as compared to siblings of individuals with cleft lip only. The author further concluded indirectly that the influence of maternal hormone imbalance may be demonstrated in the occurrence of these malformations, as well as the sex determination of offspring from other pregnancies.

Interaction of genetic and environmental factors in the etiology of cleft lip with cleft palate

It is known that etiologic heterogeneity is the greatest component in this group of birth defects (Wyszynski et al., 1996). Genetic and epidemiological studies have failed to demonstrate a single genetic mechanism in the occurrence of CLP, even while demonstrating heavy family clustering. Segregation analyses provide inconsistent evidence for the existence of a single genetic control pattern, multi-factorial inheritance, or both mechanisms involved in the development of this malformation (Maestri et al., 1997). Despite these doubts, the studies suggest that environmental factors can modify the genetic mechanism determining the occurrence of CL/P in populations (Romitti et al., 1999).

In 1989, the first reports emerged on the association between increased frequency of CL/P and the occurrence of genetic variations in the *locus* for transforming growth factors alpha and beta (TGFα/TGFβ) (Ardinger et al., 1989; Maestri, et al., 1997; Shiang et al., 1993). Genetic analysis and specific tissue expression studies support the theory that specific variants of alleles in the TGFα gene participate actively in the craniofacial development mechanism (Shiang et al., 1993). Jara et al. (1995), analyzing a racially mixed segment of the Chilean population, identified an association between TGFα and CL/P ($p < 0.014$). However, this study does not support the hypothesis that TGFα is the principal causal gene for these malformations. It can be considered a gene involved in, or which interacts with, genes in the expression of these phenotypes.

Other genes involved in morphogenesis have also been widely studied, like retinoic acid receptor alfa (RARA) (Chenevix-Trench et al., 1992), proto-oncogene BCL3 (B-cell leukemia/lymphoma-3) (Stein et al., 1995), and Msh (Drophila) homeobox homolog 1 (MSX1) (Lidral et al., 1997). On the other hand, according to Maestri et al. (1997), CLP is an expression of an uncertain inheritance pattern, with incomplete penetrance and genetic heterogeneity, both within and between populations.

Romitti et al. (1999) highlight that both the CP and CL/P phenotypes can result from the combined effects of the individual's genotype

and maternal environmental exposures. Hwang et al. (1995) demonstrated that the association between genetic variations of TGFα and cleft palate resulted from the interaction between maternal smoking and the child's genotype, in cases both with and without a family history of orofacial clefts. The OR for this interaction was 6.16 (95% CI: 1.09-43.7) for mothers who smoked up to 10 cigarettes per day and 8.69 (95% CI: 1.57-47.8) for mothers who smoked more than 10 cigarettes per day. This dose-response gradient was not statistically significant.

A population-based case-control study by Shaw et al. (1996) produced further evidence that orofacial clefts result from the interaction between genotype (particularly TGFα expression) and exogenous factors (maternal exposure to cigarette smoke from different sources). The presence of uncommon TGFα alleles in the genotype of affected children considerably increased the strength of the association between maternal smoking of more than 20 cigarettes per day and expression of the CL/P or isolated CP phenotypes (OR: 6.1; 95% CI: 1.1-36.6 for CL/P and OR: 9; 95% CI: 1.4-61.9 for CP).

A case-control study by Beaty et al. (1997) focused on the effects of maternal smoking and the occurrence of polymorphic TGFα gene markers in cases of non-syndromic orofacial clefts. This study did not show an interaction between the two; one reason may have been the small sample size limiting the statistical power to detect genetic and environmental interactions.

Romitti et al. (1999) evaluated the effects of interaction between two forms of environmental exposure (smoking and alcohol consumption) during pregnancy and the manifestation of genetic variations in gene modulators of facial morphogenesis. Only maternal smoking of more than 10 cigarettes per day showed a significant increase in the risk of developing CP in cases expressing variant alleles of TGFb3 or MSX1 (OR: 2.8; 95% CI: 1.1-6.9). As for alcohol, once again the presence of MSX1 variations characterized a statistically significant association between CLP and consumption of at least 4 doses/month (OR: 5.8; 95% CI: 1.5-27.7).

Based on these results, the occasional inconsistencies in the findings of epidemiological and genetic studies can be attributed to the heterogeneous ethnic composition, clinical characteristics, and/or environmental exposure in the target populations (Mitchell, 1996).

Discussion

Of the chemical agents evaluated and discussed above in this review, many are already known risk factors for cancer. Smoking and alcohol are heavily related to neoplasms at various anatomical sites (mouth, esophagus, larynx), pesticides and insecticides, and other chemical compounds such as organic solvents, associated with non-Hodgkin lymphoma (Rego, 1998) and pancreatic tumors (Ojajarvi et al., 2000). This observation suggests a biological plausibility deriving from the fact that the chemical agent's cellular toxicity reflects a time gradient with a possible effect in the short term (represented by fetal loss), medium term (occurrence of malformations), and long term (detection of cancer). Thus, congenital malformations could represent a sentinel event for the toxicity of certain substances associated with the development of cancer.

There is lack of agreement in the literature as to the association between hormone exposure and CLP. In the early 1990s, studies failed to demonstrate an association between hormone therapy and hypospadias, regardless of the time of use or time between conclusion of the treatment and the respective pregnancy. In addition, decreased fertility was considered a potential confounder in the association between hormone agonists and genital malformations (Källén et al., 1991a, 1991b).

An important question in the studies identifying malformations late in pregnancy or at birth is that they actually deal with the "survivors" of a cohort of malformed offspring, thus including more prevalent than incident cases (Cordier et al., 1992). Suspected or known teratogens might increase the probability not only of a birth defect but also of spontaneous abortion. However, Drushel et al. (1996) observed that mortality in the first year of life among infants with CLP not associated with other malformations is similar to that of children without birth defects, potentially reducing the occurrence of this selection bias. Other methodological issues in this research field involve prevalence estimates of congenital malformations, subject to the influence of variations in data sources, as well as the time from birth until detection and diagnosis of the defect. Such elements can introduce errors into this estimate and thus jeopardize the sensitivity of studies dealing with maternal (and/or paternal) exposure and association with the target event.

Accuracy of the response between cases and controls is a source of systematic error. A

doubt frequently arising in observational studies is whether responses by mothers of malformed children are more accurate than those of mothers of non-malformed children. Recall bias appears to be specifically associated with exposure (Werler et al., 1989). Thus, mothers of children with serious malformations tend to display better recall than others. Recall bias usually affects the recording of exposure in particular. Thus, use of medication that might play some publicly known teratogenic role may be more frequently denied than that of "harmless" drugs. According to the authors, use of controls with some type of malformation not associated with the exposure under study decreases such risks. Authors like Werler et al. (1989) and Lieff et al. (1999) support the comparison of cases of selected malformations with malformed controls. Evaluating four methods for selection of controls, Lieff et al. (1999) found that the best option is the use of controls with malformations other than the one under study. On the other hand, the use of such control groups in association studies can produce questionable results, according to Hill et al. (1988) and Prieto & Martínez-Frías (1999). The use of unhealthy controls could be useful to measure the specificity of the association between exposure and a particular defect, but it would not allow to estimate a risk ratio per se (Prieto & Martínez-Frías, 1999).

Several studies show a lack of statistical power to determine the occurrence of certain outcomes, represented by recurrent associations of reduced size with suspected human teratogens. Khoury et al. (1992) sum up the above comments when they conclude that weak associations may be influenced by five factors: potential confounders that have not been evaluated, exposure classification errors, etiologic heterogeneity of birth defects, effects of biological interactions, and differential prenatal survival. Thus, some immeasurable factors belonging to the causal chain could dilute the effects of association, resulting in smaller measures. Such factors could include the selection and recall biases discussed previously; different causal agents such as chromosome abnormalities, genetic disorders, and teratogenic exposures acting simultaneously; environmental and genetic interactions; and the action of teratogenic substances leading to the death of a portion of affected embryos.

Conclusions

Based on the studies reviewed above, we can conclude that various chemical environmental factors contribute to the occurrence of orofacial clefts, although this mechanism is not sufficiently elucidated for all suspected exposures and probably participates in an interaction phenomenon with some genes already identified. Thus, smoking and especially alcohol consumption appear to be associated with these malformations, although the measures usually obtained are small. Likewise, occupational or domestic exposures to organic solvents show biologically plausible indices. In particular, different studies have described the mechanism involved in the continuous use of anticonvulsive agents, as well as the protective effect of folic acid. As for sex hormones, the few studies conducted thus far have produced conflicting results, although the trend is to consider this exposure a risk.

Studies on the effects of environmental and occupational exposures and the occurrence of congenital malformations have gained importance with the increasing proportion of childbearing-age women in the work force. Thus, monitoring their exposure and the outcomes of their pregnancies may represent a unique opportunity to identify harmful exposures for mothers and their offspring, and could lead to future preventive measures. An important line of investigation relates to the growing population exposed to endocrine disruptors (like oral hormones) and possible malformations in individuals exposed to these substance during intrauterine life. In order to gather evidence on the safety of their use, in addition to pre-clinical trials, careful epidemiological studies should continue to be developed, reducing the percentage of unknown environmental causes associated with such outcomes.

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