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FATORES EPIDEMIOLÓGICOS, AMBIENTAIS, GENÉTICOS E DE ASSISTÊNCIA À SAÚDE RELACIONADOS ÀS FISSURAS OROFACIAIS, DESORDENS ORAIS POTENCIALMENTE MALIGNAS E CÂNCER ORAL EM POPULAÇÕES DO BRASIL E DOS ESTADOS UNIDOS

Feira de Santana, Bahia

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“Se você pode sonhar, você pode realizar.” Walt Disney

DA SILVA, Adriana Mendonça **Fatores epidemiológicos, ambientais, genéticos e de assistência à saúde relacionados às fissuras orofaciais, desordens orais potencialmente malignas e câncer oral** 2024. p.189. Tese (Doutorado em Saúde Coletiva) – Departamento de Saúde, Universidade Estadual de Feira de Santana, Bahia, 2024.

RESUMO

Introdução: As Fissuras Orofaciais são as anomalias craniofaciais mais comuns em todo o mundo, de etiologia multifatorial, são caracterizadas por alterações genéticas em mais de um gene com efeitos menores, modulada por fatores ambientais. Comorbidades maternas podem predispor a ocorrência destas fissuras. Além disso, uma intersecção etiológica entre fissuras orofaciais com lesões orais potencialmente malignas e câncer oral pode ser sugerida por fatores ambientais capazes de modular a expressão gênica em vias biológicas em comum. O objetivo desta tese foi avaliar fatores epidemiológicos, ambientais, genéticos e de assistência à saúde relacionados às fissuras orofaciais, desordens orais potencialmente malignas e câncer oral em populações do Brasil e dos Estados Unidos. **Metodologia:** Foram desenvolvidos quatro subprojetos. O subprojeto 1 consistiu em uma revisão bibliométrica da literatura para analisar tendências de pesquisas sobre a associação entre fissura orofacial e câncer. O subprojeto 2, com delineamentro transversal, investigou a associação entre fatores epidemiológicos e ambientais com a ocorrência de fissuras orofaciais, a partir da utilização bancos de dados públicos sobre nascimentos do Brasil e Estados Unidos. O subprojeto 3 foi um estudo de caso controle para analisar a associação entre comorbidades maternas e a ocorrência de fissuras orofaciais nos Estados Unidos, utilizando dados sobre nascimentos no país. O subprojeto 4, também um estudo caso controle, para investigar a associação de variantes genéticas e ambientais entre fissuras orofaciais, desordens orais potencialmente malignas e câncer oral em populações do Brasil e dos Estados Unidos, a partir de análises epidemiológicas e genéticas. **Resultados:** Com relação a tendência das pesquisas sobre a associação entre fissuras orofaciais e câncer, 70 documentos foram publicados entre 1977 e 2023, sendo a maioria deles estudos caso controle e tendo o câncer gástrico, a leucemia e o câncer de mama como os tipos mais estudados. A razão de prevalência de fissura labial e/ou palato não sindrômica no Brasil foi duas vezes maior no interior. Complicações gestacionais mostraram estar associadas a ocorrência de fissuras orofaciais nos Estados Unidos. Comparando aqueles nascidos com estas fissuras no Brasil e nos Estados Unidos, a ocorrência de fatores de risco e complicações gestacionais, foram mais evidentes nos nascidos no Brasil. Índice de massa corpórea alterada, diabetes materna e hipertensão materna foram condições associadas a um risco aumentado de desenvolvimento de fissuras orofaciais nos nascidos. Polimorfismos genéticos em genes da via WNT e fatores ambientais estiveram associados a ocorrência de desordens orais potencialmente malignas e ao carcinoma de células escamosas em populações do Brasil e dos Estados Unidos. Ainda, edentulismo aumentou o risco para desordens orais potencialmente malignas e carcinoma de células escamosas oral no Brasil. **Conclusão:** Este estudo possibilitou a identificação de fatores epidemiológicos e de assistência à saúde relacionados às fissuras orofaciais, bem como o aprofundamento do entendimento quanto a relação entre fatores ambientais e genéticos em comum para a ocorrência de fissuras orofaciais, desordens orais potencialmente malignas e câncer oral.

Palavras-chave: Fissuras orofaciais; Fenda labial; Fissura palatina; Neoplasias bucais.

DA SILVA, Adriana Mendonça **Epidemiological, environmental, genetic and health care factors related to orofacial clefts, oral potentially malignant disorders and oral cancer** 2024. p.189. Thesis (Doctorate in Public Health) – Department of Health, State University of Feira de Santana, Bahia, 2024.

ABSTRACT

Introduction: Orofacial clefts are the most common craniofacial anomalies worldwide. They have a multifactorial etiology characterized by genetic alterations in more than one gene with minor effects, modulated by environmental factors. Regarding maternal comorbidities, these may predispose to the occurrence of clefts. Furthermore, an etiological intersection between clefts with oral potentially malignant disorders and oral cancer could be suggested by environmental factors capable of modulating gene expression in common biological pathways. Thus, the aim of this thesis was to evaluate epidemiological, environmental, genetic, and healthcare-related factors related to orofacial clefts, oral potentially malignant disorders, and oral cancer in populations from Brazil and the United States. **Methodology:** Four subprojects were developed. Subproject 1 was a bibliometric review of the literature to analyze research trends on the association between orofacial cleft and cancer using a search strategy and data analysis through Biblimetrix (R Studio). Subproject 2 had a cross-sectional design to investigate the association between epidemiological and environmental factors and the occurrence of orofacial clefts, using national birth data from Brazil and the United States. Subproject 3 was a case-control study to investigate the association between maternal comorbidities and the occurrence of orofacial clefts in the United States, using national birth data. Subproject 4 will also had a case-control study design to investigate the association of genetic and environmental variants between orofacial clefts, oral potentially malignant disorders, and oral cancer in populations from Brazil and the United States, conducting genetic and epidemiological analyses. **Results:** Regarding the trend of research on the association between orofacial clefts and cancer, 70 documents were published between 1977 and 2023, with the majority being case-control studies. Gastric cancer, leukemia, and breast cancer were the most studied types. The prevalence ratio of nonsyndromic cleft lip and/or palate in Brazil was twice as high in rural areas. Gestational complications were found to be associated with the occurrence of orofacial clefts in the United States. Comparing those born with these clefts in Brazil and the United States, the occurrence of risk factors and gestational complications was more evident in those born in Brazil. Altered body mass index, maternal diabetes, and maternal hypertension were conditions associated with an increased risk of developing orofacial clefts in newborns. Single nucleotide polymorphisms in WNT pathway genes and environmental factors were associated with the occurrence of oral potentially malignant disorders and oral squamous cell carcinoma in populations from Brazil and the United States. Furthermore, edentulism increased the risk for potentially malignant oral disorders and oral squamous cell carcinoma in Brazil. **Conclusion:** This study identified epidemiological and healthcare-related factors related to orofacial clefts and deepened the understanding of the relationship between common environmental and genetic factors for the occurrence of orofacial clefts, potentially malignant oral disorders, and oral cancer.

Keywords: Orofacial clefts; Cleft lip; Cleft palate; Mouth neoplasms.

LISTA DE ILUSTRAÇÕES

LISTA DE QUADROS

LISTA DE ABREVIATURAS E SIGLAS

CCE	Carcinoma de Células Escamosas
CEP	Comitê de Ética em Pesquisa
CID-10	Classificação Internacional de Doenças versão 10
CL	*Cleft lip* (Fissura de lábio)
CL/P ou CL+P	*Cleft lip with or without palate* *(*Fissura de lábio com ou sem envolvimento de palato)
CLP	*Cleft lip with palate (*Fissura de palato)
CONEP	Comissão Nacional de Ética em Pesquisa
CP	*Cleft palate*
DRDR	*Dental Registry and DNA Repository Project* (Projeto de Registro Odontológico e Repositório de DNA)
FL	Fissura labial
FL/P ou CL+P	Fissura de lábio com ou sem envolvimento de palato
FLP	Fissura lábio e palato
FO	Fissuras orofaciais
FONS	Fissura orofacial não sindrômica
FOS	Fissura orofacial sindrômica
FP	Fissura de palato
IBGE	Instituto Brasileiro de Geografia e Estatística
NSOC	*Non syndromic orofacial cleft* (Fissura orofacial não sindrômica)
NUCAO	Núcleo de Câncer Oral
OC	Orofacial clefts
OSCC	Oral squamous cell carcinoma (Carcinoma de Células Escamosas)
REDCap	*Research Electronic Data Capture* (Pesquisa de captura eletrônica de dados)
SINASC	Sistema de Informação sobre Nascidos Vivos
SNPs	Polimorfismos genéticos de nucleotídeo único
SOC	*Syndromic orofacial cleft* (Fissura orofacial sindrômica)
STATA	*Software for Statistics and Data Science* (Software para Estatística e Ciência de Dados)
STREGA	*STrengthening the Reporting of Genetic Association Studies* (Fortalecendo o relato de estudos de associação genética)
STROBE	*Strengthening the Reporting of Observational Studies in Epidemiology* (Fortalecendo o relato de estudos observacionais em epidemiologia)
UEFS	Universidade Estadual de Feira de Santana
US	*United States* (Estados Unidos)
VSNBD	*Vital Statistics Natality Birth Data* (Estatísticas Vitais Natalidade Dados de Nascimento)
WNT	*Wingless-related integration site* (Site de integração relacionado ao Wingless)

SUMÁRIO

1. CONSIDERAÇÕES INICIAIS

1.1 Introdução

As Fissuras Orofaciais (FO) são as anomalias craniofaciais mais comuns em todo o mundo (KADIR et al., 2017; MARAZITA; MOONEY, 2004; NAGAPPAN; JOHN, 2015; NASREDDINE; EL HAJJ; GHASSIBE-SABBAGH, 2021), atingindo cerca de um a cada 700 nascidos vivos (DIXON et al., 2011). Elas representam um problema de saúde pública (AGBENORKU, 2013; ASSIS et al., 2020; FARIA, 2019; MACHADO et al., 2018; MURRAY, 2002; SANDY et al., 2020), estando relacionadas a complicações gestacionais e pós gestacionais, com efeitos a curto e longo prazo (INCHINGOLO et al., 2022). Entre as complicações fetais identifica-se o nascimento prematuro (gestacional com idade inferior a 37 semanas) antes da fase de ganho de peso fetal (abaixo de 2,500kg), que está frequentemente associada ao parto cesáreo (SHIBUKAWA et al., 2019) e maior risco de mortalidade pós-parto (MALIC et al., 2020). A relação entre as FO e a ocorrência destas complicações vem sendo pouco exploradas na literatura (WYSZYNSKI et al., 2003; WYSZYNSKI; WU, 2002).

A etiologia das FO muitas vezes é atribuída alterações genéticas em mais de um gene com efeitos menores, modulada por fatores ambientais (LESLIE; MARAZITA, 2013; REYNOLDS et al., 2020; VIEIRA, 2021a). Dessa forma, as FO configuram um transtorno multifatorial que resulta da interação complexa entre fatores genéticos, epigenéticos e exposições ambientais (ALADE; AWOTOYE; BUTALI, 2021; DIXON et al., 2011; MOSSEY et al., 2009). Essa natureza multifatorial dificulta a total compreensão etiológica das FO.

Diversos polimorfismos genéticos de nucleotídeo único (SNPs) mostraram estar associados ao aumento do risco para o desenvolvimento das FO (KHAN; PRASHANTH; SRINATH, 2020; LAMMER et al., 2004; LESLIE; MARAZITA, 2013; LETRA et al., 2009; MACHADO et al., 2018; MENEZES et al., 2009; SALEEM et al., 2019; SLAVEC et al., 2022; STUPPIA et al., 2011; VIEIRA, 2006, 2021b) e de câncer oral (HOPKINS et al., 2008; PURWANINGSIH et al., 2021; SHRIDHAR et al., 2016). Fatores ambientais e de estilo de vida, também estão associados ao desenvolvimento das FO, como a situação socioeconômica, etnia, idade dos pais, tabagismo, etilismo, ingestão de drogas/medicamentos, região geográfica, ocupação, comorbidades maternas e exposições à poluentes (GARLAND; REYNOLDS; ZHOU, 2020; KAPOS et al., 2021; NASREDDINE; EL HAJJ; GHASSIBE-SABBAGH, 2021; SAAD et al., 2014; VIEIRA, 2021c). No Brasil, estudos relacionando a prevalência de FO e fatores ambientais demostraram algumas variações nos achados, especialmente quanto a idade

materna, sexo e status socioeconômico sugerindo a necessidade de mais estudos envolvendo esta temática (ABREU et al., 2016; RODRIGUES et al., 2009; SHIBUKAWA et al., 2019; SOUSA; RONCALLI, 2017).

Com relação a comorbidades maternas, obesidade, diabetes e hipertensão, importantes problemas de saúde pública (CENTERS FOR DISEASE CONTROL AND PREVENTION, [s.d.]), com taxas crescentes na população podem resultar no aumento nas ocorrências de defeitos congênitos no feto. Contudo, a relação entre essas doenças crônicas e as FO ainda não foram suficientemente estudadas (KUTBI, 2014).

Uma vez que tanto as FO quanto o câncer são de origem multifatorial, a hipótese de que estas condições podem ocasionalmente ter uma etiologia comum (VIEIRA, 2021b) é sugerida em estudos que encontraram SNPs em comum simultaneamente associados a ambas as condições (BUI et al., 2018; MENEZES et al., 2009; TAIOLI et al., 2010; VIEIRA; KHALIQ; LACE, 2012). Dessa forma, a intersecção etiológica entre esta malformação e o câncer pode ser sugerida por fatores ambientais capazes de modular a expressão gênica em vias biológicas em comum que levariam a ambas as condições (CRUZ, 2017).

Alguns estudos têm revelado que indivíduos com FO e seus familiares apresentam um aumento do risco de desenvolver alguns tipos de canceres como leucemia, câncer de mama, cérebro, próstata, pele, fígado e intestino (BILLE et al., 2005; BUI et al., 2018; DIETZ et al., 2012; GONÇALVES et al., 2014; LETRA et al., 2009; MACHADO; POPOFF; MARTELLI-JÚNIOR, 2022; TAIOLI et al., 2010; VIEIRA, 2021b; VIEIRA; KHALIQ; LACE, 2012; YILDIRIM et al., 2012; ZHU et al., 2002). Porém, são escassos os estudos investigando a associação entre FO com Desordens Orais Potencialmente Malignas (DOPMs) (BEZAMAT et al., 2020) e câncer oral (ANDRADE FILHO et al., 2011; FREITAS et al., 2019). Além disso, esses estudos se concentraram em investigar o papel das alterações genéticas, sem considerar os fatores ambientais nessa relação, utilizando o sangue periférico para obtenção do material biológico necessário para as análises (ANDRADE FILHO et al., 2011; FREITAS et al., 2019).

A obtenção de sangue periférico, apesar de ser um método bastante utilizado em estudos envolvendo análises moleculares, é invasiva e requer profissionais capacitados para a coleta (HUANG et al., 2013; KHARE et al., 2014; NUNES et al., 2012) além da necessidade de processamento do material para extração do DNA em até uma semana após a coleta (QUINQUE et al., 2006). Por outro lado, a saliva tem se mostrado uma fonte alternativa para obtenção de DNA genômico, por ser um material de fácil obtenção, manejo e armazenamento. O método de coleta não é invasivo, minimizando risco de contaminação e desconforto, além de não necessitar de pessoas especializadas para a sua obtenção. Além disso, vários estudos têm demonstrado que

o DNA obtido da saliva apresentou pureza e rendimento semelhantes aos obtidos a partir de sangue, confirmando a importância desse material como fonte de DNA genômico (EHLI et al., 2008; HUANG et al., 2013; KHARE et al., 2014; PANDESHWAR; DAS, 2014; ZAMBUZZI, 2012). Adicionalmente, a saliva apresenta um grande número de células descamadas do epitélio oral garantindo a concentração de DNA necessária para as análises moleculares (POLGÁROVÁ; BEHULIAK; CELEC, 2010; QUINQUE et al., 2006). As amostras de saliva, diferente do sangue, podem ser congeladas por longos períodos mostrando-se uma alternativa bastante viável para experimentos de discriminação alélica de polimorfismos (RAMOS et al., 2019).

Desta forma, sob a hipótese de uma possível associação entre fissuras orofaciais com desordens orais potencialmente malignas e câncer oral, o objeto deste estudo foi a avaliação de fatores epidemiológicos, ambientais e genéticos relacionados às fissuras orofaciais, desordens orais potencialmente malignas e câncer oral em populações do Brasil e dos Estados Unidos.

1.2 Revisão de literatura

1.2.1 Fissuras orofaciais

1.2.1.1 Aspectos epidemiológicos relacionados às fissuras orofaciais

As FO ocorrem devido a uma separação incompleta entre as cavidades nasal e oral entre a quarta e a décima segunda semana do desenvolvimento embrionário craniofacial (BERNHEIM et al., 2006; MARAZITA; MOONEY, 2004; MOSSEY et al., 2009). Uma falha parcial ou total durante a formação dos lábios ou palato primário, entre a quarta e a oitava semana, resultará no desenvolvimento de fissura de lábio (FL) ou de fissura de lábio e palato (FLP). Por sua vez, falhas durante a formação do palato secundário (palato duro e mole), entre a quinta e a décima segunda semana, levará a ocorrência de fissura de palato isolada (FP) (NASREDDINE; EL HAJJ; GHASSIBE-SABBAGH, 2021). Dessa forma, as fissuras de lábio com ou sem envolvimento de palato (FL/P) demonstram ter origem embriológica distinta da FP (MOSSEY et al., 2009).

As FO são as anomalias craniofaciais mais comuns em todo o mundo (KADIR et al., 2017; MARAZITA; MOONEY, 2004; NAGAPPAN; JOHN, 2015; NASREDDINE; EL HAJJ; GHASSIBE-SABBAGH, 2021). Elas atingem mais de 10 milhões de vidas e estima-se um novo

caso a cada três minutos no mundo (SANDY et al., 2020). Sua prevalência mundial é de 1/700 nascidos vivos (DIXON et al., 2011; LESLIE; MARAZITA, 2013; MOSSEY; MODELL, 2012), com variações geográficas, socioeconômicas, raciais e entre os sexos (MOSSEY et al., 2009).

Em relação às variações socioeconômicas e geográficas, o Canadá possui uma das maiores taxas de FO do mundo, com aproximadamente 1/892 nascidos vivos (MALIC et al., 2020). Nos Estados Unidos, as FO representam o terceiro defeito congênito mais comum, ocorrendo em aproximadamente 1/1.000 nascidos vivos (MAI et al., 2019), a exemplo do Estado do Texas, com uma prevalência de FO de 16,4/10.000 (MESSER et al., 2010). Em países de baixa e média renda a prevalência de FO é de aproximadamente 1/730 nascidos vivos (KRUPPA et al., 2021). Estudos transversais com abrangência nacional realizados no Brasil encontraram uma prevalência de FO variando entre 3,6 e 5,1/10.000 nascidos, a depender do período analisado (ABREU et al., 2016; RODRIGUES et al., 2009; SHIBUKAWA et al., 2019; SOUSA; RONCALLI, 2017) (Quadro 1). Algum destes ainda se propuseram a avaliar a relação entre as taxas de prevalência e algumas variáveis socioeconômicas, de atenção materno infantil e biológicas. Estudos também foram realizados visando descrever o perfil clínico epidemiológico dessa malformação em diferentes localidades no país, incluindo o estado da Bahia e a cidade de Feira de Santana (ALVES; MOURA; FREITAS, 2019; MOURA, 2014) (Quadro 2).

Quadro 1 – Estudos transversais sobre a prevalência das fissuras orofaciais no Brasil

Local (Autor, ano)	Objetivo	Desfecho	Unidade de análise	Ajustes	Análise estatística
Brasil (RODRIGUES et al., 2009)	Investigar a prevalência de FO entre 1998-2002 e correlacionar a fatores socioeconômicos.	Prevalência de 3,6/10.000. Ausência de correlação com indicadores socioeconômicos (IDH, Índice de Gini, renda per capita, mortalidade infantil)	27 capitais brasileiras	-	Correlação de Pearson.
Brasil (SOUSA; RONCALLI, 2017)	Investigar a prevalência de FO entre 2009-2013 e correlacionar ao número de procedimentos cirúrgicos realizados pelo SUS.	Prevalência de 5,8/10.000. Presença de correlação com número de procedimentos cirúrgicos por região.	5 regiões brasileiras e seus Estados	-	Correlação de Pearson.

Quadro 1 – Estudos transversais sobre a prevalência das fissuras orofaciais no Brasil (Continuação)

Local (Autor, ano)	Objetivo	Desfecho	Unidade de análise	Ajustes	Análise estatística
Brasil (ABREU et al., 2016)	Investigar a prevalência e estimar a tendência temporal da prevalência de FO entre 2000-2014.	Prevalência de 4,8/ 10.000. Tendência de aumento na prevalência de FO nas regiões menos desenvolvidas do país.	5 regiões brasileiras	Idade materna; Número de consultas pré-natal	Regressão binomial negativa.
Brasil (SHIBUKAWA et al., 2019)	Analisar a tendência e fatores associados às FO e verificar associações com fatores materno infantis entre 2005-2016.	Prevalência de 5,1/10.000. Idade materna (+), estado civil (+), duração da gestação (+), número de consultas de pré natal (+), tipo de parto (+), sexo (+), raça/cor (+), Apgar (+) e peso ao nascer (+).	5 regiões brasileiras	-	*Odd ratio.* Regressão polinomial

(+): associação estatisticamente mais significante.

Quadro 2 - Estudos observacionais descritivos sobre o perfil epidemiológico das FO no Brasil

Local (Autor, ano)	Objetivo	Fonte de dados	População	Desfecho
Belo Horizonte (DI NINNO et al., 2011)	Traçar o perfil epidemiológico das FO de um centro especializado de BH	Centro de referência para tratamento de FO entre 2005-2008.	1.219 prontuários	Maior ocorrência de FLP, no sexo masculino, no interior.
Maringá (COSTA; FARAH; JACOBUCCI, 2013)	Determinar a prevalência de FO em Maringá e região.	Centro de referência para tratamento de FO entre 1988-2011.	424 prontuários	Maior ocorrência de FLP, no sexo masculino e etnia branca, em área urbana.
Paraná (MATOS et al., 2020)	Analisar o perfil epidemiológico das FO de crianças atendidas em um centro de referência.	Centro de referência para tratamento de FO entre 2013-2017.	116 prontuários	Maior ocorrência FLP, no sexo masculino, na região urbana.
Amazonas (ALARCÓN; SÁ, 2017)	Apresentar aspectos epidemiológicos das FO dos pacientes atendidos em um centro de referência.	Centro de referência para tratamento de FO entre 2014-2016.	477 prontuários	Maior ocorrência de FLP, no sexo masculino.
Sergipe (TEIXEIRA, 2015)	Determinar a distribuição, e caracterização e dos casos de FO em Sergipe.	Centro de referência para tratamento de FO entre 2003-2013.	669 prontuários	Maior ocorrência de FLP, no sexo masculino, parto vaginal, etnia branca, no interior.
Feira de Santana (ALVES; MOURA; FREITAS, 2019)	Descrever o perfil epidemiológico das FO em Feira de Santana.	Centro de referência para tratamento de FO entre 2008-2013.	18 indivíduos	Maior ocorrência FLP, no sexo feminino, na região urbana, mães com 26-34 anos e nível médio de escolaridade.
Bahia (MOURA, 2014)	Descrever o perfil clínico-epidemiológico das FO na Bahia.	Centro de referência para tratamento de FO entre 2008-2013.	319 prontuários	Maior ocorrência de FLP, no sexo feminino, na região urbana, mães com 16-25 anos e nível fundamental.
Bahia (FREITAS et al., 2013)	Descrever os casos de FO na Bahia, quanto ao sexo, etnia, tipo de FO e localização geográfica.	Centro de referência para tratamento de FO entre 2000-2010.	1752 prontuários	Maior ocorrência de FLP, no sexo masculino, etnia preta e parda, no interior.
Bahia (VIENA et al., 2017)	Descrever fatores ambientais e sociodemográficos de FO de três centros de referência na Bahia.	Três centros de referência para tratamento de FO.	135 indivíduos	Maior ocorrência de FLP no sexo feminino, etnia negra, na região urbana, mães com com 15-25 anos.

Quadro 2 - Estudos observacionais descritivos sobre o perfil epidemiológico das FO no Brasil (Continuação)

Local (Autor, ano)	Objetivo	Fonte de dados	População	Desfecho
São Luís (RODRIGUES et al., 2014)	Descrever as características dos nascidos vivos com malformação congênita em São Luís	SINASC para NV com malformações entre 2002-2011 em São Luís.	876 casos	Maior ocorrência de malformação em termos, com peso normal, de gestação única e parto cesáreo, do sexo masculino, cor parda, Apgar satisfatórios, mães com 20-34 anos, solteiras, com 8 a 11 anos de estudo e com 4-6 consultas de pré-natal.
Rio Grande do Norte (FIGUEIRÊDO et al., 2011)	Analisar os aspectos epidemiológicos das FO em crianças nascidas no Rio Grande do Norte.	SINASC para NV com FO entre 2000-2005 no Estado do Rio Grande do Norte	155 casos	Maior ocorrência FP, no sexo masculino, com peso normal, parto vaginal, termo, cor preta.
Paraíba (ANDRADE et al., 2018)	Avaliar a distribuição da ocorrência das FO	SINASC para NV com FO entre 2011-2015 em seis cidades da Paraíba.	109 casos	Maior ocorrência de FLP no sexo masculino, com peso normal ao nascer, no interior, mães com 20-29 anos.
Região Nordeste (CAVALCANTI et al., 2019)	Avaliar a distribuição da ocorrência de FO na região nordeste do Brasil.	SINASC para NV com FO entre 2011-2016 no Nordeste.	902 casos	Maior ocorrência de FLP no sexo masculino, não brancas e com peso normal ao nascer.

NV: Nascidos Vivos; FO: Fissura Orofacial; FLP: Fissura de lábio e palato; FP: Fissura de palato; BH: Belo Horizonte

As FO apresentam diferentes distribuições entre as raças e sexos (SILVA et al., 2008). Alguns estudos descreveram maior ocorrência de FO na raça/cor branca (DI NINNO et al., 2011; TEIXEIRA, 2015), enquanto outros, na raça/cor preta parda (FIGUEIRÊDO et al., 2011; FREITAS et al., 2013; RODRIGUES et al., 2014; VIENA et al., 2017). Quanto ao sexo, as FL/P afetam preferencialmente o sexo masculino (2:1 homem/mulher), enquanto as FP são mais frequentes entre mulheres (1:2 relação homem/mulher) (DIXON et al., 2011).

1.2.1.2 Classificação das fissuras orofaciais

A identificação precisa do fenótipo das FO é crucial para entender tanto a epidemiologia quanto a etiologia dessa malformação (MONLLEÓ et al., 2013; SANDY et al., 2020). Assim, algumas classificações para as fissuras têm sido propostas na literatura (MCBRIDE et al., 2016; NASREDDINE; EL HAJJ; GHASSIBE-SABBAGH, 2021) (Quadro 2).

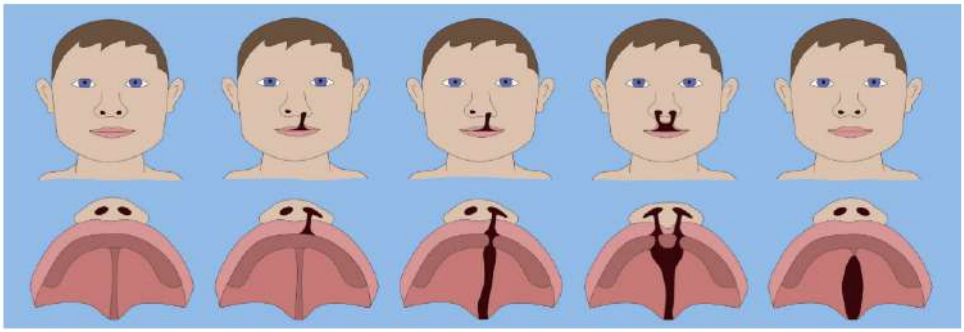
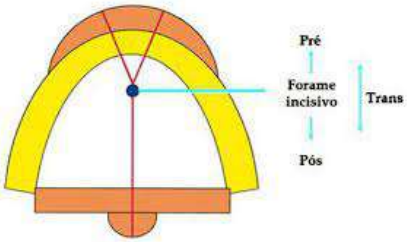
A classificação básica fundamenta-se na origem embriológica dividindo em fissura labial (FL) sem ou com envolvimento do palato (FL/P) e fissura exclusivamente palatina (FP) (BERNHEIM et al., 2006; MOSSEY et al., 2009) (Quadro 3.A). Dentro desta classificação, existem as subdivisões. A FL pode ser unilateral ou bilateral, completa, quando está associada à fissura alveolar e envolvendo as narinas, ou incompleta com um leve entalhe no lábio superior. O espectro da FP varia de uma fenda submucosa a uma fenda completa do palato primário e secundário (NASREDDINE; EL HAJJ; GHASSIBE-SABBAGH, 2021).

Uma classificação comummente adotada no Brasil é a de Spina (SPINA et al., 1972) (Quadro 3.B) que utiliza o forame incisivo como referência anatômica. Ela fornece uma divisão das fissuras em quatro grupos: grupo I ou pré-forame (completo, incompleto); grupo II ou transforame; grupo III ou pós-forame (completo, incompleto); e grupo IV que envolve fissuras raras da face que não estão relacionadas ao forame incisivo.

As FO podem ser classificadas também com base na presença ou ausência de qualquer anomalia física e/ou de desenvolvimento adicional em mais de uma região ou órgão do corpo como, respetivamente, fissuras orofaciais sindrômicas (FOS) ou não sindrômicas (FONS) (DIXON et al., 2011; MOSSEY et al., 2009; NASREDDINE; EL HAJJ; GHASSIBE-SABBAGH, 2021; SALEEM et al., 2019; SANDY et al., 2020; SLAVEC et al., 2022) (Quadro 3.C). As FOS incluem os defeitos congênitos múltiplos (fissura com um ou mais defeitos maiores associados, sem diagnóstico de uma síndrome reconhecível) e as síndromes (com base no diagnóstico do registro individual) (IPDTOC WORKING GROUP, 2011; WORLD HEALTH ORGANIZATION, 2006). As FONS são as mais frequentes (GARLAND; REYNOLDS; ZHOU, 2020; REYNOLDS et al., 2020; WORLEY; PATEL; KILPATRICK, 2018), correspondendo a aproximadamente 70 a 80% das FL/P (MOSSEY; MODELL, 2012) e 50% de casos de FP (DIXON et al., 2011; YU et al., 2017).

Ainda existe a classificação preconizada pela Classificação Internacional de Doenças, que é um sistema usado para codificar patologias em todo o mundo (WORLD HEALTH ORGANIZATION (WHO), 2022). Esta classificação é extremamente útil para a análise epidemiológica da saúde geral entre grupos populacionais, fornecendo informações relacionadas à prevalência e incidência de doenças e problemas de saúde específicos. Em sua versão 10 (CID-10), as FO são classificadas no Capítulo XVII, referente a “Malformações congênitas, deformidades e anomalias cromossômicas” (Q00 a Q99) com códigos: Q35 para FP, Q36 para FL e Q37 para FLP (Quadro 3.D).

Quadro 3 – Principais sistemas de classificação das fissuras orofaciais

<table>
<tr><td>(A)
Quando a origem embriológica*</td><td colspan="2"> 
Normal CL FLP unilateral FLP bilateral FP</td></tr>
<tr><td>(B)
Quanto a classificação de Spina**</td><td colspan="2"> 
</td></tr>
<tr><td rowspan="3">(C)
Quanto a presença ou ausência de anomalias associadas***</td><td>Fissuras orofaciais não sindrômicas (FONS)</td><td>FO sem nenhuma malformação adicional;
FO com até três defeitos menores associados.</td></tr>
<tr><td>Fissuras orofaciais sindrômicas (FOS)</td><td>FO com quarto ou mais defeitos menores;
FO com diagnóstico de síndrome;
FO como defeito congênito múltiplo.</td></tr>
<tr><td rowspan="3">(D)
Quanto a codificação da CID-10</td><td>Q35 – Fenda palatina</td><td>Q35.1: fenda palatina dura.
Q35.3: fenda palatina mole.
Q35.5: fenda palatina com fenda palatina mole.
Q35.9: fenda palatina não especificada.</td></tr>
<tr><td>Q36 – Fenda labial</td><td>Q36.0: lábio leporino bilateral.
Q36.1: lábio leporino mediana.
Q36.9: lábio leporino unilateral.</td></tr>
<tr><td>Q37 – Fenda labial e palatina</td><td>Q37.0: fenda palatina com lábio leporino bilateral.
Q37.1: fenda palatina com lábio leporino unilateral.
Q37.2: fenda palatina com lábio leporino bilateral.
Q37.3: fenda palatina com lábio leporino unilateral.
Q37.4: fenda palatina dura e mole com lábio leporino bilateral.
Q37.5: fissura de palato duro e mole com fissura labial unilateral.
Q37.8: fenda palatina com lábio leporino bilateral, não especificado.
Q37.9: fenda palatina com lábio leporino unilateral, não especificado.</td></tr>
</table>

Fonte: Adaptado de *SANDY et al., 2020; **ARAÚJO, 1999; SPINA et al., 1972; ***AQUINO et al., 2014; FERRARI-PILONI, 2021; MONLLEÓ et al., 2015.

1.2.1.3 Etiologia das fissuras orofaciais

As FO podem variar quanto a etiologia, gravidade e severidade (LESLIE; MARAZITA, 2013; MARAZITA; MOONEY, 2004; NASREDDINE; EL HAJJ; GHASSIBE-SABBAGH, 2021; SILVA et al., 2018). Quanto a sua etiologia, as FO podem ocorrer a partir de um modo de herança monossômica, multifatorial ou cromossômica (VIEIRA, 2021a). Ainda, podem ter uma origem epigenética, quando fatores ambientais influenciam a expressão gênica, sem que

necessariamente haja uma alteração genética, levando ao desenvolvimento das FO (LESLIE, 2022).

As fissuras sindrômicas são frequentemente causadas por herança monossômica, onde alteração em um único gene (mutações e deleções) com efeito maior é responsável pela ocorrência da fissura durante o desenvolvimento embrionário (VIEIRA, 2021a). Em cerca de 75% destes casos, as fissuras fazem parte do fenótipo de alguma síndrome com causa genética conhecida, atualmente identificadas em mais de 300, a exemplo da Síndrome de Van der Woude (LESLIE; MARAZITA, 2013; MARAZITA; MOONEY, 2004; VIEIRA, 2021a). Os outros 25% incluem casos de FOS com etiologia desconhecida, caracterizando os defeitos congênitos múltiplos (DIXON et al., 2011; IPDTOC WORKING GROUP, 2011; LESLIE; MARAZITA, 2013; MONLLEÓ et al., 2015).

Por sua vez, a etiologia das FONS muitas vezes não é atribuída por uma única mutação facilmente identificável, mas sim a alterações genéticas em mais de um gene com efeitos menores, modulada por fatores ambientais (LESLIE; MARAZITA, 2013; REYNOLDS et al., 2020; VIEIRA, 2021a). Dessa forma, as FONS configuram-se um transtorno multifatorial que resulta da interação complexa entre fatores genéticos, epigenéticos e exposições ambientais (ALADE; AWOTOYE; BUTALI, 2021; DIXON et al., 2011; MOSSEY et al., 2009) (Figura 1).

Fatores não modificáveis (biológicos)	**Fatores modificáveis (pré concepção)**	**Fatores modificáveis (pré e pós concepção)**
1. Polimorfismos genéticos	7. Consanguinidade	13. Peso
2. Etnia	8. Idade dos pais na concepção	14. Estado nutricional
3. Sexo do nascido	9. Nível socioeconômico	15. Doenças maternas agudas e crônicas
4. História familiar de FO	10. Nível educacional	16. Estresse psicofísico
5. Paridade	11. Local de residência	17. Drogas lícitas e ilícitas
6. Alterações epigenéticas	12. Clima	18. Exposição a poluentes e/ou pesticidas

Figura 1 - Principais fatores de risco para as fissuras orofaciais
Fonte: Adaptado de INCHINGOLO et al., 2022.

1.2.2 Fatores biológicos relacionados às fissuras orofaciais

Fatores de risco biológicos para as FONS são considerados fatores não modificáveis uma vez que não são possíveis de ser alterados por ações educativas em saúde. Estes incluem polimorfismos genéticos, alterações epigenéticas, sexo do nascido, etnia e história familiar de FO e paridade (INCHINGOLO et al., 2022) (Figura 1).

Variações em genes que ocorrem devido a alterações em uma única base nucleotídea criam SNPs entre os indivíduos, que quando ocorrem em genes codificadores, podem ter o potencial de gerar um aminoácido diferente do esperado, alterar a expressão gênica e resultar no desenvolvimento de determinada característica ou fenótipo (BULL, 2013), a exemplo das fissuras.

Estudos utilizando uma variedade de abordagens já foram realizados para a identificação de genes que contribuem para a ocorrência de FONS (ALADE; AWOTOYE; BUTALI, 2021; CRUZ, 2017; LESLIE, 2022; LESLIE et al., 2017; LUDWIG et al., 2012; MARAZITA, 2012; MUKHOPADHYAY et al., 2021; SALEEM et al., 2019; SLAVEC et al., 2022; STUPPIA et al., 2011; VIEIRA, 2006; YU et al., 2017). Esses estudos encontraram alterações genéticas em vários genes e loci genéticos, incluindo genes que codificam fatores de crescimento (TGFA e TGFB3) e fatores de transcrição (SUMO1, MSX1, TBX22, FOXE1, PAX7 e MAFB), genes envolvidos no metabolismo de xenobióticos (CYP1A1, GSTM1 e NAT2), genes que participam do metabolismo nutricional (MTHFR e RARA) e genes envolvidos na resposta imune (PVLR1 e IRF6). Nesse contexto, merecem destaque as alterações em genes da via WNT (*Wingless-related integration site*) que tem papel crucial no crescimento e diferenciação celular, sendo críticos para o desenvolvimento craniofacial (ALADE; AWOTOYE; BUTALI, 2021).

A predisposição genética para FONS é dependente da etnia e do sexo, variando entre as diferentes populações (DIXON et al., 2011). A influência do sexo do nascido para a ocorrência de FO apresenta uma correlação específica com o tipo de fissura. Estudos tem constado que o sexo masculino apresenta um risco elevado para o desenvolvimento de FL/P e FL (2:1 homem/mulher), enquanto FP é mais frequente em mulheres (1:2 relação homem/mulher) (BERG et al., 2015; DIXON et al., 2011; FIGUEIREDO et al., 2015; INCHINGOLO et al., 2022; SHIBUKAWA et al., 2019). Uma possível explicação para esse comportamento seria que o palato feminino conclui sua formação uma semana mais tarde que o masculino (VAN AALST; KOLAPPA; SADOVE, 2008).

Quanto a etnia, diferenças étnicas tem o potencial de influenciar na ocorrência de FONS. De forma geral, asiáticos e ameríndios apresentam maior frequência de FO, seguidos por

brancos/europeus (1/500 nascidos vivos) e população africana (1/2.500 nascidos vivos) (DIXON et al., 2011; SHIBUKAWA et al., 2019; SILVA et al., 2018). Mas estes achados variam na literatura, o que pode estar relacionado a diferenças na forma de aferição e categorização de grupos étnicos e em variações na classificação das fissuras. No Brasil, diferenças ainda podem estar relacionadas a miscigenação da população, formada por ameríndios, europeus e africanos, que pode influenciar na determinação do genótipo (CAVALCANTI et al., 2019; FIGUEIRÊDO et al., 2011; FREITAS et al., 2013). Uma revisão sistemática (MACHADO et al., 2018),descrevendo os marcadores genéticos de risco para FONS na população brasileira, sugeriu evidências de associação entre risco aumentado para fissuras relacionados a IRF6, 8q24 ou 8MTHFR. Já entre populações de descendência européia, uma meta-análise (SLAVEC et al., 2022) encontrou outras variantes (*GRHL3, VAX1, TGFA, FOXE1, ABCA4, NOG, GREM1, AXIN2, DVL2, WNT3A e WNT5A*) com alto potencial de biomarcadores para fissuras.

A história familiar de FO é considerada um fator crítico para o desenvolvimento de fissuras, com achados descritos na literatura (INCHINGOLO et al., 2022; LY et al., 2017; NOOROLLAHIAN et al., 2015; SILVA et al., 2018). Taxas aumentadas de recorrência familiar FO podem ser explicadas pela maior probabilidade de compartilhamento de alelos semelhantes por meio de descendência (SILVA et al., 2018).

A paridade (número de fetos por gestação) também pode ser um fator importante para o desenvolvimento de FO. Achados revelam que mães com maior paridade pode ter maior risco de terem filhos com malformações congênitas (ALMEIDA; ALMEIDA; PEDREIRA, 2015; LUO et al., 2013). Embora os mecanismos subjacentes a esta associação permaneçam obscuros, tem sido sugerido que alterações fisiológicas da mãe durante a gravidez múltipla podem ter efeitos adversos nos micronutrientes e subsequentemente aumentar o risco de FO (GOLALIPOUR M J et al., 2012; VU et al., 2022).

Alterações em processos epigenéticos, como metilação do DNA ou modificação de histonas, bem como dos microRNAs não codificantes que regulam a expressão gênica, também são influenciados por fatores ambientais e podem ser responsáveis pelo desenvolvimento das FO (GARLAND et al., 2020) e câncer (MOHAMMED; SPRINGFIELD; DAS, 2012).

Apesar das evidências, as variantes genéticas para FONS expressam um baixo risco e não explicam a herdabilidade total estimada (FARIA, 2019; VISSCHER; HILL; WRAY, 2008) levando a acreditar de que existem fatores genéticos desconhecidos (um grande número de variantes de baixo risco e variantes raras de grande efeito) ou outros mecanismos, enfatizando

a influência de importantes fatores ambientais compartilhados pela família relacionados à ocorrência dessa malformação (CRUZ, 2017; FARIA, 2019).

1.2.3 Fatores ambientais relacionados às fissuras orofaciais

Estudos epidemiológicos vem demonstrando o papel dos fatores ambientais para o desenvolvimento de FONS (ANGULO-CASTRO et al., 2017; CHESHMI et al., 2020; ESHETE et al., 2020; GARLAND; REYNOLDS; ZHOU, 2020; NASREDDINE; EL HAJJ; GHASSIBE-SABBAGH, 2021; RAUT et al., 2019; REGINA ALTOÉ et al., 2020; SATO et al., 2021; XU et al., 2018). Esses fatores, sozinhos ou combinados aos fatores genéticos, são comumente responsáveis por cerca de 60% dos casos de FONS (SALEEM et al., 2019).

Os fatores de risco ambientais relacionados à ocorrência de FO são considerados como fatores modificáveis, pois podem ser prevenidos antes e/ou depois da concepção por meio de ação educativas em saúde (Figura 1) (INCHINGOLO et al., 2022).

1.2.3.1 Fatores de risco ambientais modificáveis (pré concepção) e as fissuras orofaciais

O grupo de fatores de risco ambientais modificáveis pré concepcão inclui consanguinidade, idade dos pais na concepção, nível socioeconômico e educacional, local de residência e clima (Figura 1). Esses resultados podem impactar significativamente a prevenção de iniquidades em saúde (INCHINGOLO et al., 2022).

A idade dos pais pode atuar tanto como fator de risco como fator de proteção para as FONS. Estudos tem encontrado que mães mais velhas (idade superior a 35 anos) (DAI et al., 2010; FIGUEIRÊDO et al., 2011; LEI et al., 2013; MESSER et al., 2010) ou muito novas (idade inferior a 19 anos) (MOURA, 2014; VIENA et al., 2017) apresentaram maior risco do nascimento de crianças com FO (BERG et al., 2015; DE CARVALHO et al., 2016; FIGUEIREDO et al., 2015; SHIBUKAWA et al., 2019), enquanto idades entre 26 e 35 anos teriam um efeito protetor (JAMES et al., 2020). Uma possível explicação para estes achados são mudanças cumulativas nos gametas ao longo da vida como resultado de exposições ambientais ou alterações cromossômicas, ao processo de envelhecimento do útero tornando-o menos seletivo para embriões defeituosos, ao fato da placenta de mulheres mais velhas ser mais permeável aos agentes teratogênicos, ao uso de medicamentos ao longo da vida, a prevalência de doenças crônicas em idosos, a características socioeconômicas, além do acesso e qualidade dos cuidados de saúde (HERKRATH et al., 2012). Quanto à idade paterna, achados sugerem

que o risco de ocorrência de FO na criança aumenta com o avanço idade (BERG et al., 2015; HERKRATH et al., 2012). Porém a literatura ainda não tem um consenso sobre a relação entre a idade dos pais e as FO (AHMED SAKRAN et al., 2022; DE CARVALHO et al., 2016; LY et al., 2017).

Fatores socioeconômicos são apontados como relacionados a problemas de saúde e se apresentam como fatores contribuintes para o aparecimento de anomalias congênitas (ALI; HAMID, 2019; ANTUNES et al., 2011; DONKOR; PLANGE-RHULE; AMPONSAH, 2007). Estudos tem demonstrado que pais com baixa condição socioeconômica têm maior probabilidade de gerar filhos com FO (CARMICHAEL; MA; SHAW, 2009; CLARK et al., 2003; ESCOFFIÉ-RAMIREZ; ET AL., 2010; LUPO et al., 2015; NEHRA et al., 2021; SMILLIE et al., 2015). Além disso, iniquidades socioeconômicas, desigualdades nas condições de vida e trabalho e aspectos sociais relacionados a um baixo nível de coesão social geram uma maior susceptibilidade a exposição de fatores ambientais e que podem influenciar a situação de saúde por meio de diversos mecanismos biológicos (NOTTERMAN; MITCHELL, 2015).

Por sua vez, os fatores relacionados à assistência pré-natal e atenção materno infantil (ANDRADE et al., 2018; ANGULO-CASTRO et al., 2017; CAVALCANTI et al., 2019) são fatores que desempenham um papel na situação de saúde/doença e têm sido trazidos na literatura como possivelmente relacionados ao desenvolvimento das fissuras. Os avanços nas técnicas de imagem pré-natal facilitaram a identificação no pré-natal das FO e tem papel fundamental na preparação das famílias, na qualidade dos cuidados pós-natais e de vida geral (SREEJITH et al., 2018). Além disso, a assistência pré-natal no primeiro trimestre diminuiu o risco de FO, provavelmente devido à prevenção de deficiências de vitaminas e/ou minerais e identificação de exposições prejudiciais que podem atrapalhar o desenvolvimento orofacial embrionário (VU et al., 2022). A frequência mínima de seis consultas de pré-natal esteve associada a menores taxas de internação em Centros Intensivos neonatais e a mortalidade neonatal nas primeiras 72 horas de vida no Brasil (RODRIGUES et al., 2022).

O nível de escolaridade é um importante indicador do grau de inserção social materna e tem sido relacionado com os desfechos da gravidez, do parto e do recém-nascido, pois repercute no acesso, acompanhamento e supervisão de saúde, nos cuidados com a gestação e redução de seus eventos adversos (SILVESTRIN et al., 2017). Indivíduos com baixo nível educacional, quando comparados àqueles com nível superior, tendem a fumar mais, a consumir alimentos menos saudáveis e a usar menos frequentemente suplementação vitamínica durante o período gestacional (DVIVEDI; DVIVEDI, 2012), aumentando probabilidade de gerar filhos com FO (FIGUEIREDO et al., 2015). Estima-se que para cada ano que se aumenta a escolaridade da

mãe e do pai o risco de terem filhos com FO diminui, respectivamente, 19% e 16% (GONZÁLEZ-OSORIO et al., 2011).

Poucos estudos se propuseram a investigar a relação entre local de residência e FO (INCHINGOLO et al., 2022). No Brasil, observou-se um predomínio de FONS entre indivíduos residentes de cidades do interior do país (COUTINHO et al., 2009; DI NINNO et al., 2011; FREITAS et al., 2013; TEIXEIRA, 2015). Considerando os tipos de FO, a frequência de FL/P foi maior em áreas do interior, enquanto FP em áreas costeiras (VIEIRA et al., 2021). Com relação a desigualdades entre áreas rurais e urbanas, evidências sugerem que mães que vivem em áreas rurais têm maior probabilidade de gerar crianças com FONS do que mães que vivem em áreas urbanas nos Estados Unidos (KAPOS et al., 2021; MESSER et al., 2010). Indícios de que a maioria dos pais de crianças FO residem em regiões rurais, levam os pesquisadores a concluir que esse defeito congênito é mais comum em pessoas de posição socioeconômica mais baixa (NOOROLLAHIAN et al., 2015). Uma maior exposição materna aos pesticidas em áreas rurais, principalmente por razões ocupacionais poderia também justificar uma maior vulnerabilidade dessa população a FO nos nascidos (DAHIRI et al., 2021; SPINDER et al., 2017; UEKER et al., 2016).

O estudo das tendências sazonais de anomalias congênitas leva em consideração diversos fatores, como intensidade da luz ultravioleta, temperatura, radiação, exposição a químicos agrícolas, ciclo de doenças infecciosas, disposição de alimentos, nutrição materna, entre outros (KROST; SCHUBERT, 2006). A prevalência da ocorrência de FO em certa estação do ano pode ajudar no entendimento do papel do ambiente sobre esse defeito congênito. Em alguns países do hemisfério norte há um pico de nascimentos de crianças com FO nos meses de maio/outubro no Canadá e abril na Finlândia, sugerindo que a concepção destas crianças no inverno rigoroso destes países, poderia estar associado a baixa ingestão de folatos na dieta neste período. Os resultados dos estudos variam amplamente, possivelmente devido as diferenças geográficas, climáticas e socioeconômicas, onde alguns autores não encontraram qualquer associação da sazonalidade com o desenvolvimento de FO, considerando a data de concepção (LUIZ; RODRIGUES; DEMARCO, 2022). Os achados também não são conclusivos em relação a temperaturas mais quentes, onde apesar destas levarem a piores resultados na gravidez, alguns autores não encontraram nenhum risco aumentado estatisticamente significativo de malformações do tubo neural (SOIM et al., 2018).

1.2.3.2 Fatores de risco ambientais modificáveis (pré e pós concepção) e as fissuras orofaciais

O grupo de fatores de risco ambientais modificáveis pré e pós concepção inclui preferencialmente fatores relacionados a condições e hábitos de estilo de vida maternos (KRAPELS et al., 2006) como peso alterado, deficiências nutricionais durante a gestação, drogas lícitas e ilícitas, estresse, doenças maternas agudas e crônicas e infecções virais (INCHINGOLO et al., 2022).

O papel do índice de massa corporal (IMC) materno para a ocorrência de desfechos no feto vem sendo estudado. Mães obesas (25-29,9 kg/m^2) ou com sobrepeso (>30kg/m^2) tiveram um risco maior de ter bebês com FO do que as mulheres com IMC normal (18,5 e 25kg/m^2) (IZEDONMWEN; CUNNINGHAM; MACFARLANE, 2015; KUTBI et al., 2017; KUTBI, 2014; RANKIN et al., 2010; STOTHARD et al., 2009). Uma possível explicação seria que a obesidade materna pode causar hiperglicemia, hiperinsulinemia, estresse oxidativo, inflamação sistêmica e deficiências institucionais que podem comprometer o desenvolvimento do feto, predispondo a ocorrência de malformações (CORREA et al., 2008; KING, 2006). De forma inversa, poucos autores mostraram uma correlação entre baixo peso materno (<18,4kg/m^2) e FO (RANKIN et al., 2010; WALLER et al., 2007).

Os fatores nutricionais associados a ocorrência das FONS envolvem nutrientes que desempenham papéis críticos durante a embriogênese, a exemplo dos folatos (GARLAND; REYNOLDS; ZHOU, 2020; JAYARAJAN; NATARAJAN; NAGAMUTTU, 2019), retinóides (GARLAND; REYNOLDS; ZHOU, 2020; JOHANSEN et al., 2008; NASREDDINE; EL HAJJ; GHASSIBE-SABBAGH, 2021) e zinco (HOZYASZ et al., 2009). Outros nutrientes, incluindo vitamina B12, B1, B6, ácido piridóxico e homocisteína também foram indicados com uma possível associação com a ocorrência de FO (HAO et al., 2015; MURRAY; WEHBY, 2010; REGINA ALTOÉ et al., 2020).

Sobre o papel das drogas na etiologia das FONS, pode-se dividi-las em duas famílias. A primeira inclui as drogas ilícitas que têm efeitos deletérios à saúde e podem levar a FO, como a cocaína (MARKOV; JACQUEMYN; LEROY, 2003). O segundo grupo é composto por medicamentos, como Amoxicilina, Fenitoína, Oxprenolol, Thietilperazina, Carbamazepina, Oxitetraciclina e Anticonvulsivantes (Fenobarbital, Trimetadiona, Valproato e Dilantina), que aumentam o risco de FO em bebês nascidos de mães em tratamento com estes medicamentos (HOLMES, 2004; PUHÓ et al., 2007). Além disso, foi encontrada associação entre o uso de

corticóides sistêmicos no primeiro trimestre de gestação e a ocorrência de FO (PRADAT et al., 2003).

Os fatores de risco para as FONS mais comumente estudados são o consumo de tabaco e bebidas alcoólicas. Uma recente revisão sistemática e meta-análise confirmou a associação entre tabagismo materno e a ocorrência de FO, encontrando um Odds de 1,42 (IC 95% 1,27-1,59) e fração atribuível à população de 4% (IC 95% 3%-5%) (FELL et al., 2022). Essa associação provavelmente está relacionada aos vários teratógenos presentes na fumaça do tabaco, como hidrocarbonetos aromáticos, dioxinas, monóxido de carbono, pesticidas e substâncias pesadas, que podem causar hipóxia fetal, uma condição conhecida por induzir as FO. Com relação ao consumo de bebidas alcoólicas, existem várias hipóteses para os mecanismos teratológicos do etanol relacionados ao antagonismo da sinalização do ácido retinóico, a alterações epigenéticas e ao estresse oxidativo (GARLAND; REYNOLDS; ZHOU, 2020).

Quanto a doenças maternas e o risco de FONS, duas merecem destaque: diabetes e hipertensão (CHESHMI et al., 2020; CORREA et al., 2008; INCHINGOLO et al., 2022; KUTBI, 2014). O *diabetes mellitus* é uma doença metabólica caracterizada por hiperglicemia. Quando esta ocorre durante o período da gravidez, ela é denominada como *diabetes mellitus* gestacional. Esta condição se resolve sozinha após o parto, mas mulheres afetadas tem maior risco de terem recém-nascidos com FO. Ainda, a associação da *diabetes mellitus* gestacional com obesidade pode triplicar o risco de o feto desenvolver FO (TRINDADE-SUEDAM et al., 2016). Embora os mecanismos subjacentes às associações entre diabetes e anomalias congênitas não sejam completamente compreendidos, parece que a hiperglicemia durante a embriogênese poderia aumentar o estresse oxidativo, alterações epigenéticas, hipóxia e apoptose, contribuindo para danos no DNA e um maior risco de malformações congênitas, como as FO (ERIKSSON; CEDERBERG; WENTZEL, 2003; KOZMA, 2019). Quanto a hipertensão, a presença desta patologia antes da gravidez também esteve associada a um aumento risco de desenvolver fissuras (TRINDADE-SUEDAM et al., 2016).

Ainda, há evidências sobre a exposição a patógenos e o risco de FO, a exemplo de algumas infecções virais (varicela, rubéola, vírus Epstein-Barr, herpesvírus, citomegalovírus e influenza), bacterianas (micoplasma, clamídia e sífilis) e protozoária (Toxoplasma gondii), no entanto, mais estudos são necessários para elucidar tais associações (GARLAND; REYNOLDS; ZHOU, 2020).

1.2.4 Complicações gestacionais e pós gestacionais relacionadas as fissuras orofaciais

Ass FO como um problema de saúde pública (AGBENORKU, 2013; ASSIS et al., 2020; FARIA, 2019; MACHADO et al., 2018; MURRAY, 2002; SANDY et al., 2020), causa um impacto negativo não apenas nos indivíduos portadores desta malformação, mas também em suas famílias e na sociedade, com gastos substanciais em saúde (MARAZITA; MOONEY, 2004; MICHALSKI et al., 2015; MOSSEY et al., 2009; NASREDDINE; EL HAJJ; GHASSIBE-SABBAGH, 2021; SALEEM et al., 2019; SILVA et al., 2018). As complicações geradas pelas FO podem ter repercussões gestacionais e pós gestacionais, com efeitos a curto e longo prazo.

As complicações gestacionais podem ser comumente divididas em maternas e fetais (INCHINGOLO et al., 2022), podendo surgir em qualquer estágio da gestação, durante o trabalho de parto ou no pós-parto. As complicações maternas incluem aborto espontâneo, interrupção eletiva da gravidez devido a identificação pré-natal de malformação congênita, hiperêmese gravídica e anemia por deficiência de folato e vitamina B12 (AHMED SAKRAN et al., 2022; CHESHMI et al., 2020; DIEN et al., 2018). Entre as complicações fetais identifica-se o nascimento prematuro (idade gestacional inferior a 37 semanas) antes da fase de ganho de peso fetal (<2,500kg), que está frequentemente associada ao parto cesáreo (SHIBUKAWA et al., 2019), piores condições fisiológicas nos primeiros minutos de vida (Apgar menor que 7) e maior risco de mortalidade pós-parto (FARINHAS, 2017; MALIC et al., 2020).

Nascimentos prematuros ocorreram em cerca de 13,4 milhões de gestações em 2020 no mundo (WORLD HEALTH ORGANIZATION, 2023). Estudos realizados nos Estados Unidos e na Coréia do Sul revelaram uma associação entre FO e prematuridade (RP=1,83) (SHEHAN et al., 2021; RYU et al., 2022). Embora essa relação não seja completamente compreendida, esses resultados reforçam a hipótese de que fatores maternos que contribuem para a prematuridade são importantes fatores de risco para o desenvolvimento de fissuras, ajudando a melhorar a estratificação de risco, o aconselhamento materno e as intervenções (SHEHAN et al., 2021).

Crianças prematuras e com baixo peso ao nascer (<2.500g) tendem a apresentar taxas mais elevadas de problemas motores, cognitivos, comportamentais e problemas emocionais, aumentando a necessidade de apoio educacional e médico (FAN; PORTUGUEZ; NUNES, 2013). Crianças com FO apresentaram maior risco de nascer com baixo peso (DEROO; GAUDINO; EDMONDS, 2003). Uma razão seria que gestações de crianças com malformações são mais suscetíveis a problemas de saúde e a fatores de risco que contribuem para um menor

peso ao nascer. Dessa forma, melhorar o acesso aos cuidados pré-natais para mulheres grávidas com diagnóstico pré-natal de FO pode desempenhar um papel importante na prevenção do baixo peso ao nascer (NYARKO et al., 2013).

A cesariana é a cirurgia mais realizada nos Estados Unidos, com mais de um milhão de partos cesáreos realizados a cada ano (SUNG; MAHDY, 2023). Em relação aos países de renda média e baixa, a taxa de cesarianas continua a aumentar (BETRÁN et al., 2021). Estudos realizados no Brasil encontraram que crianças com FO tendem a nascer preferencialmente por parto cesáreo (FIGUEIRÊDO et al., 2011; RODRIGUES et al., 2014). Este resultado é em parte esperado, uma vez que as anomalias congênitas são uma das indicações do feto para cesariana (BARBER et al., 2011; SUNG; MAHDY, 2023). Contudo, esse achado pode também estar associado ao fato do Brasil apresentar um elevado número de partos cesáreos, sendo o segundo país com maior taxa de cesarianas no mundo (BETRÁN et al., 2021). A preocupação global em otimizar a realização de cesarianas se dá devido ao risco de potenciais complicações relacionadas à mortalidade e morbidade materna e perinatal por essa cirurgia (BETRÁN et al., 2021).

As condições fisiológicas (cor, frequência cardíaca, reflexos, tônus muscular e respiração) do nascido nos primeiros minutos de vida é avaliada pelo Índice Apgar (SIMON; HASHMI; BRAGG, 2023). Nascidos que apresentaram índice de Apgar menor que sete no 1º e 5º minutos revelaram mais chances de evoluírem para óbito dos que os bebês com escore acima de oito, sugerindo a necessidade da oferta de assistência de qualidade no momento do parto para diminuição dos riscos de morte dos bebês com FO (FARINHAS, 2017).

Complicações pós gestacionais a curto prazo incluem os efeitos deletérios produzidos na infância como dificuldades de fala e audição, problemas alimentares e dentários (CHRISTENSEN et al., 2004). A longo prazo, estas incluem problemas psicológicos, relacionados a própria deformidade facial e ao estresse psicológico da criança e suas famílias. As FO podem ainda influenciar no nível de escolaridade, por contribuir para faltas escolares recorrentes e dificuldades de integração social ao longo da vida (YAZDY et al., 2007; NASREDDINE; EL HAJJ; GHASSIBE-SABBAGH, 2021).

Além disso, esses indivíduos têm uma expectativa de vida reduzida em cerca de 10 anos (CHRISTENSEN et al., 2004), possivelmente devido a maiores taxas de morbidade e mortalidade em comparação com a população geral (AGBENORKU, 2013; ASSIS et al., 2020; FARIA, 2019; MACHADO et al., 2018; MOSSEY et al., 2009; MURRAY, 2002; SANDY et al., 2020), com um maior risco de desenvolvimento de problemas cardiovasculares (CHRISTENSEN et al., 2004), suicídio (CHRISTENSEN et al., 2004) e câncer (BILLE et al.,

2005; DIETZ et al., 2012; GONÇALVES et al., 2014; JINDAL; VIEIRA, 2012; LESLIE; MARAZITA, 2013; LETRA et al., 2009; MACHADO; POPOFF; MARTELLI-JÚNIOR, 2022; POPOFF et al., 2013; YILDIRIM et al., 2012; ZHU et al., 2002).

Indivíduos com FO necessitam de tratamento e acompanhamento multidisciplinar desde o nascimento até a idade adulta, envolvendo cirurgiões plásticos, ortodontistas, fonoaudiólogos, entre outros profissionais de saúde, o que aumenta os custos em saúde a curto e longo prazo (NASREDDINE; EL HAJJ; GHASSIBE-SABBAGH, 2021). Em média, o tratamento de uma criança com FO requer seis cirurgias, 20 anos de atendimento hospitalar, cinco anos de tratamento ortodôntico e terapia fonoaudiológica contínua ("Pittsburgh Orofacial Cleft Studies", 2022; SANDY et al., 2020).

Um estudo em um banco de dados nacional identificou diversas disparidades raciais/étnicas no tratamento de FL e FP primárias, com redução do tratamento de reparo destas fissuras ao longo do tempo em crianças não-brancas. Os pacientes asiáticos foram significativamente mais propensos a ter atraso no reparo destas fissuras, de acordo com as diretrizes da Associação Americana de Fissura Craniofacial-Palatina. Esses achados ressaltam a necessidade de compreender melhor as disparidades no tempo de reparo da fissura e nos resultados pós-operatórios (MULLEN et al., 2023). Existem diferenças significativas nas probabilidades de atrasos, complicações, internações hospitalares prolongadas e total de cobranças entre pacientes com FO de diferentes raças/etnias. Esforços de defesa para melhorar as disparidades na atenção a saúde infantil nos primeiros anos de vida pode subsequentemente melhorar os resultados dos tratamentos (PECK, 2022).

No Brasil, a atenção às pessoas com FO é financiada pelo governo por meio do Sistema Único de Saúde desde 1993 (MONLLEÓ et al., 2013; MONLLEÓ; MOSSEY; GIL-DA-SILVA-LOPES, 2009). Mas apesar dos avanços nos últimos anos, a qualidade da assistência varia substancialmente entre os estados brasileiros (MONLLEÓ; MOSSEY; GIL-DA-SILVA-LOPES, 2009), ainda persistindo os desafios e as disparidades no tratamento de pacientes com FO, com regiões sem centros credenciados pelo Sistema Único de Saúde, o que eleva os custos com deslocamento, levando muitas famílias a interromper o seguimento do tratamento (MONTEIRO et al., 2018; SOUSA; RONCALLI, 2017).

1.2.5 Fissuras orofaciais e câncer oral

O câncer envolve um grupo de doenças caracterizadas pela divisão descontrolada de células com potencial de sofrer metástase. O câncer de cavidade oral representa um problema de saúde pública, com taxas de incidência, mortalidade e sobrevida bastante variável em todo o mundo (RAHMAN et al., 2020). Segundo informações do Instituto Nacional de Câncer, o número de novos casos de câncer da cavidade oral esperados para o Brasil no ano de 2023 foi de 10.900 casos em homens e de 4.200 em mulheres ("Ministério da Saúde Instituto Nacional de Câncer José Alencar Gomes da Silva Ministério da Saúde Instituto Nacional de Câncer", 2023).

A maioria dos cânceres derivados do epitélio da mucosa da cavidade oral, faringe e laringe são conhecidos coletivamente como câncer de cabeça e pescoço (JOHNSON et al., 2020). Histopatologicamente, o câncer de cavidade oral são frequentemente do tipo carcinoma de células escamosas (CCE), que correspondem a mais de 90% dos casos da doença (GUPTA; JOHNSON; KUMAR, 2016). O CCE por vezes está associado ou é precedido por DOPMs que representam um grupo de lesões e condições caracterizadas por um risco variável de desenvolver câncer de lábio e cavidade oral (AGUIRRE-URIZAR; LAFUENTE-IBÁÑEZ DE MENDOZA; WARNAKULASURIYA, 2021; REIBEL et al., 2017).

A apresentação clínica das DOPMs geralmente consistem em uma placa branca (leucoplasia oral) ou vermelha (eritroplasia), com textura lisa (homogênea) ou áspera (não homogênea), não destacável (RANGANATHAN; KAVITHA, 2019). A Organização Mundial de Saúde ainda inclui outros fenótipos na classificação das DOPMs, representados por líquen plano oral; fibrose submucosa oral; queratose actínica (queilite actínica); lesões palatais por fumo reverso; lúpus eritematoso oral; disceratose congênita; lesão liquenóide oral e doença do enxerto-versus-hospedeiro (WARNAKULASURIYA et al., 2021).

O tecido epitelial que reveste a cavidade oral pode apresentar alterações morfológicas em que a combinação de anormalidades citológicas e distúrbios arquiteturais compreendem os critérios histopatológicos para o diagnóstico de displasias epiteliais orais, as quais demonstram maior tendência a sofrer transformação maligna para o CCE quando comparado com o epitélio normal (BRENNAN et al., 2007). A progressão para o desenvolvimento do CCE pode ser inciada com uma hiperplasia de células epiteliais da mucosa que pode ser seguida por uma série de alterações genéticas que resultam em displasias epiteliais orais (WETZEL; WOLLENBERG, 2020) Estas displasias podem evoluir ou não de forma gradual (leve, moderada, severa) para o carcinoma *in situ* (grau mais severo de displasia) e finalmente para o carcinoma invasivo (JOHNSON et al., 2020; RANGANATHAN; KAVITHA, 2019; REIBEL

et al., 2017). O diagnóstico histopatológico das displasias orais pode representar um indicativo de transformação maligna, embora nem todas as lesões displásicas progridam necessariamente para o câncer (BRENNAN et al., 2007; WARNAKULASURIYA, 2009; WARNAKULASURIYA et al., 2021).

O desenvolvimento do câncer de cavidade oral é resultado da interação entre fatores ambientais e aqueles relacionados a suscetibilidade genética do hospedeiro (MCMAHON; CHEN, 2003). As alterações genéticas promovidas por esses agentes carcinogênicos e infeções virais são responsáveis por iniciar o processo da carcinogênese, a partir alterações em vias normais de crescimento, divisão e apoptose celular que predispõem a displasias, o surgimento de algumas DOPMs e evolução para o CCE (LIU, 2018).

Alguns fatores ambientais têm sido apontados como preditores para ocorrência de câncer de cavidade oral, como a idade (GUNTINAS-LICHIUS et al., 2010), a infeção por papiloma vírus humano (ADAMS; WISE-DRAPER; WELLS, 2014; PYTYNIA; DAHLSTROM; STURGIS, 2014), hábitos de estilo de vida como o abuso no consumo do tabaco e de bebidas alcoólicas (AMARASINGHE et al., 2013; HASHIBE et al., 2009; HINDLE et al., 2000), além dos fatores de privação social (RYLANDS; LOWE; ROGERS, 2016), onde grupos menos privilegiados, com menor status socioeconômico, apresentam maior exposição a fatores de risco podendo resultar em um maior número de casos desta doença nesta população (CONWAY et al., 2008). Tais fatores podem atuar isoladamente ou em conjunto no desenvolvimento da neoplasia (JOHNSON et al., 2020).

SNPs têm sido estudados como potenciais fatores prognósticos e preditivos em uma variedade de cânceres incluindo câncer gástrico (TOFFOLI; CECCHIN, 2007), pulmonar (HEIST et al., 2007), colorretal (MARTINEZ-BALIBREA et al., 2007), de mama (STERNLICHT et al., 2006) e esôfago (IZZO et al., 2007). Estudos mostraram também que polimorfismos em genes envolvidos no metabolismo e na resposta imune como *CTLA4* (rs231775 e rs4553808), *Il10* (1082A>G), *P450 1A1* (CYP1A1; Ile462Val) e glutationa S-transferase μ1 (GSTM1; polimorfismo nulo) (CADONI et al., 2012; FANG et al., 2018; JOHNSON et al., 2020; NIU et al., 2015; WANG et al., 2016b), e em genes responsáveis pela regeneração e diferenciação celular, a exemplo de SNPs em genes *WNT11* e *AXIN2* na via WNT (PURWANINGSIH et al., 2021), estiveram associados ao aumento do risco de CCE de cabeça e pescoço. Por sua vez, o desenvolvimento de DOPMs também mostrou estar relacionado a polimorfismos em genes da via WNT (*WNT3, AXIN2)* (BEZAMAT et al., 2020; EJAZ; GHAFOOR, 2019; NADER; GATAA; MOHAMMAD, 2017) e a mais seis SNPs localizados nos genes *GSTM1* (nulo), *CCND1* (G870A), *MMP3* (-1171; região promotora), *TNFa* (-308;

rs800629), *XPD* (códon 751) e *Gemin3* (rs197412) (SHRIDHAR et al., 2016). Além das alterações genéticas, as alterações epigenéticas também têm um papel na etiologia do CCE, principalmente pela hipermetilação do DNA e a expressão desregulada de determinados genes (JOHNSON et al., 2020).

Assim, tanto as FO como o câncer são condições resultantes de contribuições ambientais e genéticas significativas, caracterizando o modo de herança multifatorial, na qual o fenótipo ocorre devido a expressão de mais de um gene, com efeitos relativamente pequenos, e sendo esta expressão modificada pelo ambiente (VIEIRA, 2021a). Dessa forma, evidências têm investigado a associação de FO e câncer em diferentes populações baseando-se na hipótese de que ambas compartilham fatores ambientais em comum capazes de provocar uma modificação na atividade genética, contribuindo para o risco de desenvolvimento destas doenças (ALTMANN; HALLIDAY; GILES, 1998; BENUSIGLIO et al., 2013; BILLE et al., 2005; BJØRGE et al., 2008; BLOT; STILLER; KINNIER-WILSON, 1980; BOTTO et al., 2013; BRESLOW; BECKWITH, 1982; BUI et al., 2018; CARDOSO et al., 2018; CAROZZA et al., 2012; DIAS et al., 2020; DIETZ et al., 2012; FISHER et al., 2012; GOLD et al., 1994; GONÇALVES et al., 2014; JINDAL; VIEIRA, 2012; KLUIJT et al., 2012; LIMA; SILVÉRIO; OLIVEIRA, 2013; MANN et al., 1993; MARTELLI et al., 2014; MENEZES et al., 2009; MERTENS et al., 1998; NAROD et al., 1997; NISHI et al., 2000; OBERMAIR et al., 2019; STEINWACHS et al., 2000; TAIOLI et al., 2010; VIEIRA; KHALIQ; LACE, 2012; YILDIRIM et al., 2012; YU et al., 2001; ZACK; ADAMI; ERICSON, 1991; ZHU et al., 2002).

Estudos têm demonstrado que indivíduos nascidos com FO são pelo menos seis vezes mais propensos a desenvolver câncer, e em seus parentes de primeiro e segundo grau o risco de desenvolver esta doença é três vezes maior (VIEIRA; KHALIQ; LACE, 2012). Ainda, de forma inversa, indivíduos em tratamento contra o câncer ou que sobreviveram ao mesmo demonstraram estar mais propensos a ter história familiar de FO (JINDAL; VIEIRA, 2012; TAIOLI et al., 2010).

Uma recente revisão sistemática, com metanálise, foi realizada com o objetivo de resumir as evidências clínicas sobre a relação entre câncer e FONS, envolvendo 17 estudos de caso-controle, 13 estudos transversais e 3 relatos de casos, abrangendo uma população de 206.096 pacientes de vinte países. Destes, 0,35% dos pacientes com câncer (IC 95%: 0,0% a 1,1%; I2 = 86%), 3,0% dos familiares de pacientes com câncer (IC 95%: 1,19% a 5,46%; I2 = 55%), e 0,26% dos controles (IC 95%: 0,0% a 0,83%; I2 = 87%) possuíam FONS. Entre os estudos que examinaram a prevalência de câncer, 2,4% (IC 95%: 0,0% a 19,3%; I2 = 99%) dos pacientes com FONS, 15,4% dos familiares de pacientes com FONS (IC 95%: 2,0% a 37,6 %; I2 = 99%)

e 5,3% dos controles (IC 95%: 0,0% a 22,8%; I2 = 99%) possuíam câncer. Embora não tenha sido observada relação entre o risco de câncer em pacientes com FONS e o risco de FONS em pacientes com câncer, houve uma associação para um risco aumentado de câncer em parentes de pacientes com FONS (OR: 9,96, IC 95%: 1,55 a 63,99; p = 0,01) e associação significativa para o risco de FONS em familiares de pacientes com leucemia (OR: 9,31; IC 95%: 1,13 a 76,67; p = 0,03) (MACHADO; POPOFF; MARTELLI-JÚNIOR, 2022).

Com relação aos fatores ambientais, alguns deles como o ambiente social, escolaridade, status socioeconômico, hábitos de estilo de vida e comportamentais (consumo de tabaco, bebidas alcoólicas, acesso nutricional limitado) são determinantes sociais de saúde que a curto prazo podem favorecer a ocorrência de distúrbios de desenvolvimento, a exemplo das FO, e a médio prazo, influenciar no risco de câncer, considerando que, frente a uma condição adversa, o processo genético/epigenético do feto é programado para selecionar qual órgão receberá a energia para crescimento e desenvolvimento adequado e, assim, danos causados nesse estágio podem resultar em FO (MANCILLA et al., 2020). Por sua vez, o câncer tem risco influenciado por fatores ambientais aos quais o indivíduo é exposto durante a vida (BANAUDHA; KUMAR; VERMA, 2018).

As FO e câncer são condições que apresentam fenótipos diferentes, porém o mecanismo biológico regulado por determinado gene é o mesmo. Dessa forma, estudos genéticos têm apoiado uma relação etiológica entre as FO e câncer sustentando a hipótese de que SNPs que ocorrem em genes de vias relacionadas a proliferação e diferenciação celular, a exemplo da via sinalização WNT, poderiam contribuir para um aumento na susceptibilidade a ocorrência de FO durante o desenvolvimento embrionário e a ocorrência de câncer em algum estágio da vida (PURWANINGSIH et al., 2021; VIJAYAN et al., 2018), incluindo o câncer de cavidade oral (ANDRADE FILHO et al., 2011; FREITAS et al., 2019).

A via de sinalização WNT é fundamental para a regulação de vários processos biológicos como divisão, migração e determinação do destino celular, motilidade, polaridade, eixo primário formação e organogênese, processos estes importantes não somente durante desenvolvimento embrionário, mas também para a regeneração e integridade celular em adultos (KOMIYA; HABAS, 2008). Dessa forma, uma desregulação nessa via vem sendo relacionada a defeitos congênitos, a exemplo das FO, defeitos esqueléticos e cânceres (ANDRADE FILHO et al., 2011; BUGTER; FENDERICO; MAURICE, 2021; KARIM et al., 2004; LIU; MILLAR, 2010; MENON, 2017; VIJAYAN et al., 2018; ZHAN; RINDTORFF; BOUTROS, 2017). Ainda, vários mecanismos moleculares relacionados a essa via vem sendo mencionados quanto

à transformação maligna de lesões orais (EJAZ; GHAFOOR, 2019; KOMIYA; HABAS, 2008; LIU; MILLAR, 2010; NADER; GATAA; MOHAMMAD, 2017; REYES et al., 2019).

A via WNT é capaz de estimular várias cascatas de transdução de sinal intracelular, incluindo a via canônica (dependente de Wnt/β-catenina) e a via não canônica (independente de β-catenina) (Figura 2). A via canônica compreende uma família de ligantes WNT (a exemplo do Wnt2, Wnt3a, Wnt3b, Wnt8, Wnt10), receptores e co-receptores que estão associados ao sinal transdução através da célula e irão induzir várias respostas intracelulares. Na presença de um ligante Wnt, o Axin se associa ao receptor de lipoproteína fosforilada (LRP), o complexo de destruição (Axin, APC e GSK3) se desfaz e a degradação da b-catenina é inibida, permitindo que ela se acumule no citoplasma e se transloque para o núcleo, onde ela interage com fatores de transcrição (TCF) para iniciar a transcrição de genes alvo. Porém, na ausência de um ligante Wnt, a b-catenina citoplasmática sofre uma degradação proteassômica pelo complexo de destruição e a transcrição de genes alvo no núcleo é inibida. Na via não canônica, os ligantes (Wnt5a, Wnt4, Wnt11 e outros) atuam por ligação a repectores na via Wnt/Ca^{2+} (aumentando os níveis intracelulares de Ca2+ e ativando a proteína kinase C) ou na via Wnt/PCP (ativando a N-terminal kinase), em ambas resultando na ativação do gene alvo no núcleo (EJAZ; GHAFOOR, 2019; ZHANG et al., 2022).

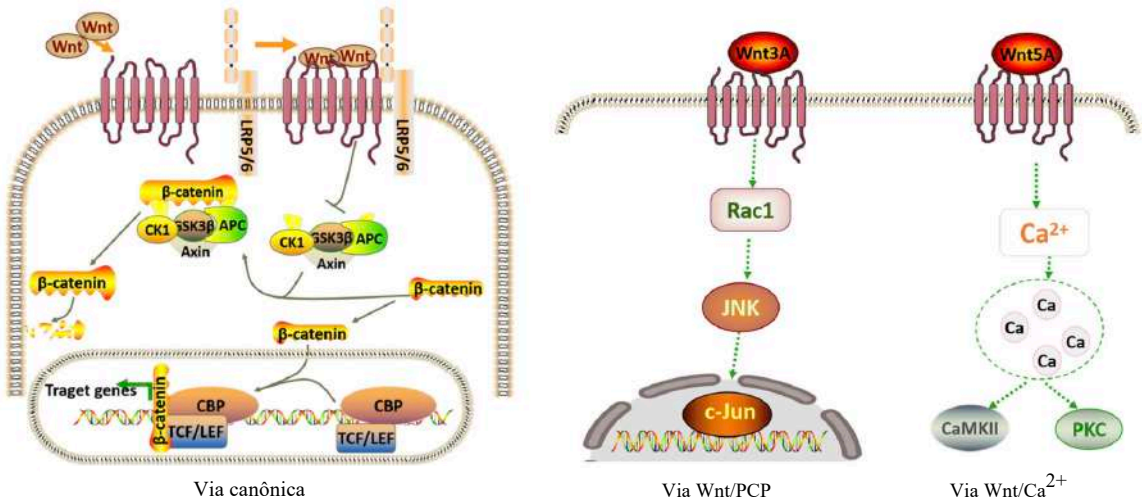


Figura 2 – Via de sinalização WNT
Fonte: ZHANG et al., 2022.

A via WNT vem sendo estudada em relação a alguns tipos de cânceres, com destaque para o câncer de mama e leucemia (MACHADO; POPOFF; MARTELLI-JÚNIOR, 2022). Com relação ao câncer oral, foram encontrados dois estudos caso-controle que investigaram a associação de câncer oral com SNPs em genes da via WNT comumente associados a ocorrência

de FO (Quadro 4). Os achados destes estudos sugerem que alterações genéticas nessa via, mais especificadamente SNPs nos genes *WNT11, GSK3B* e *AXIN2*, poderiam aumentar a suscetibilidade de um indivíduo desenvolver CCE, explicando uma possível relação entre a ocorrência de FO e câncer oral (ANDRADE FILHO et al., 2011; FREITAS et al., 2019), uma vez que alterações relacionadas a migração celular, proliferação, transdiferenciação e apoptose são consideradas intimamente associadas à ocorrência de FO e eventos comumente correlacionados com o câncer (WANG et al., 2016). Porém, estes estudos não conseguiram reunir dados sobre variáveis ambientais nas análises. Dessa forma, uma futura compreensão da interação entre fatores genéticos e ambientais, pode auxiliar no melhor entendimento da relação entre estas doenças (ANDRADE FILHO et al., 2011; CRUZ, 2017; GARLAND; REYNOLDS; ZHOU, 2020).

Quadro 4 – Estudos que investigaram a associação entre fissuras orofaciais e câncer oral

Autor, ano, País	**Objetivo**	**Amostra (caso/ controle)**	**Grupo caso**	**Desfecho**	**Análise de dados**	**Genotipagem (SNPs)**	**Ajustes para potenciais confundidores**	**SNPs (gene) estatisticamente associados a FO e câncer oral**
Andrade Filho et al., 2011, Estados Unidos	Investigar se genes associados à FONS também estão associados ao câncer oral.	413 (188/225)	Câncer oral	FONS (FL/P)	Diferenças nas frequencias dos alelos	32 SNPs em 9 genes da via WNT	-	rs9879992 (*GSK3B*) rs1533767 (*WNT11*) rs3923087 (*AXIN2*)
Freitas et al., 2019, Brasil	Avaliar a associação de SNPs e FONS em pacientes com câncer oral e de mama.	209 (79/130) câncer oral e 280 (159/121) câncer de mama	Câncer oral e Câncer de mama	FONS (FL/P)	Diferenças nas frequencias dos alelos; Regressão logística.	5 SNPs em 5 genes da via WNT	Idade, sexo, tabagismo e consumo de álcool (para câncer oral) e idade (para câncer de mama)	rs9879992 (*GSK3B*) rs1533767 (*WNT11*)

SNPs = polimorfismos genéticos; FONS = fissuras orofaciais não sindrômicas; FL/P = fissura de lábio com ou sem envolvimento de palato;

Além disso, várias lesões orais caracterizadas como DOPMs, como eritroplasia, leucoplasia oral e líquen plano oral também compartilham alterações em mecanismos moleculares regulados por esses genes da via WNT (EJAZ; GHAFOOR, 2019). SNPs no gene *AXIN2* demonstraram estar associados a leucoplasia oral (BEZAMAT et al., 2020). O aumento da expressão citoplasmática de AXIN2 em leucoplasia oral demonstrou está fortemente associado à transformação mesenquimal epitelial que favorece a progressão e transformação maligna dessas lesões, funcionando assim como um oncogene de progressão tumoral (ZHANG et al., 2017). O aumento da expressão citoplasmática e nuclear de WNT3 demonstrou contribuir para alteração displásica do líquen plano oral (NADER; GATAA; MOHAMMAD, 2017). O

aumento da expressão nuclear da b-catenina foi relacionado a uma maior gravidade e potencial de transformação maligna de displasias epiteliais orais apresentadas clinicamente como eritroplasia e leucoplasia (REYES et al., 2015).

Outros fenótipos orofaciais que demonstraram ter associação a SNPs em genes da via WNT foram agenesia dentária (CARDOSO et al., 2018; KÜCHLER et al., 2013; LETRA et al., 2009), cárie dentária e doença periodontal (ZHANG et al., 2022).

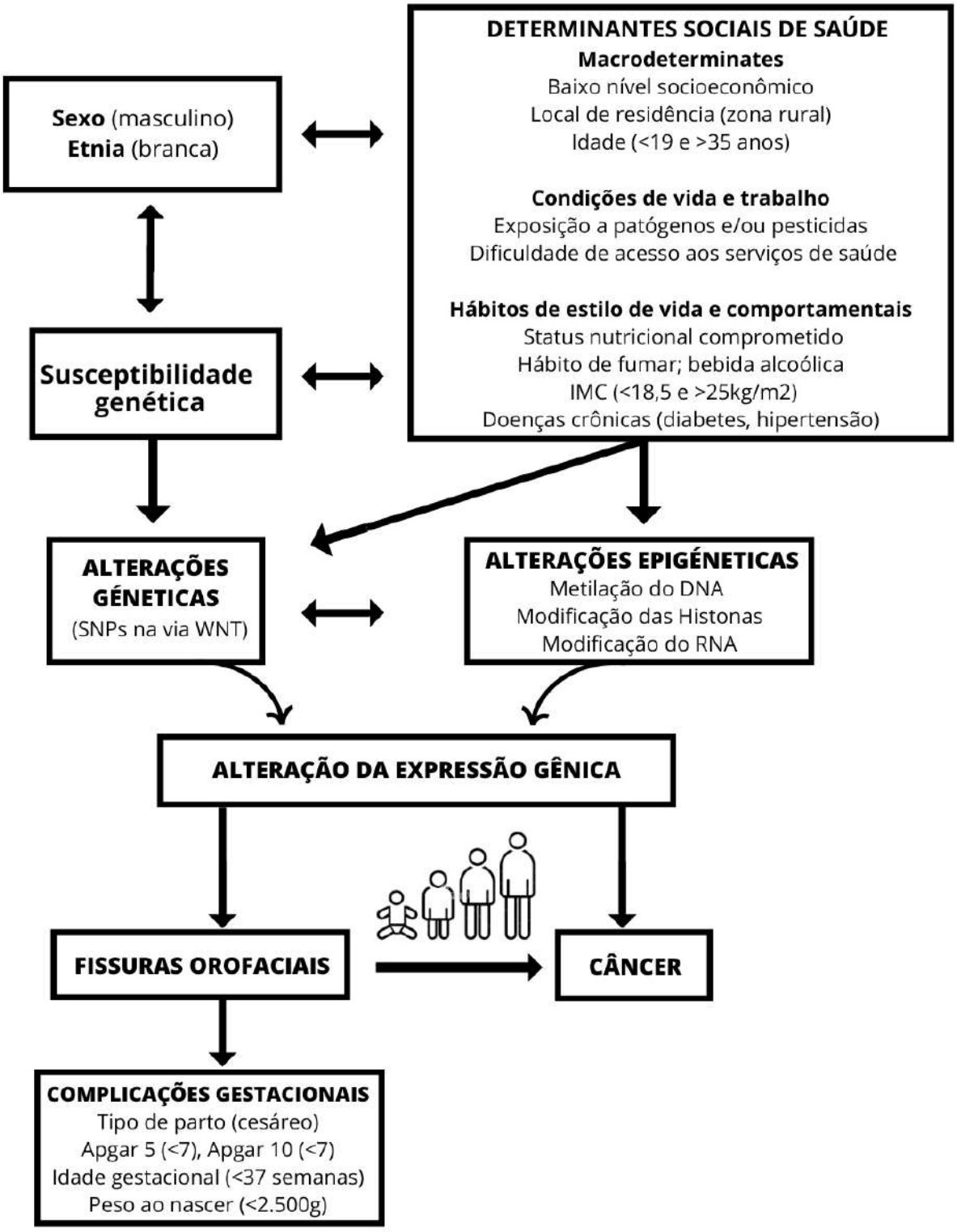


Figura 3 – Modelo teórico-conceitual para associação entre fatores ambientais, fatores genéticos, fissuras orofaciais e câncer
Fonte: Próprio autor

3. OBJETIVOS

3.1 Objetivo geral

- Avaliar fatores epidemiológicos, ambientais, genéticos e de assistência à saúde relacionados às fissuras orofaciais, desordens orais potencialmente malignas e câncer oral em populações do Brasil e dos Estados Unidos.

3.2 Objetivos específicos

1. Analisar a tendência das pesquisas sobre a associação entre fissura orofacial e câncer quanto a implicações do crescimento de publicações, cocitação, copalavras e redes de autoria utilizando indicadores bibliométricos e visualização de redes;
2. Investigar a associação entre fatores epidemiológicos e ambientais com a ocorrência de fissuras orofaciais no Brasil e Estados Unidos;
3. Investigar a associação de variantes genéticas e ambientais entre fissuras orofaciais, desordens orais potencialmente malignas e câncer oral em populações do Brasil e dos Estados Unidos;
4. Analisar associação entre a presença de comorbidades maternas e o nascimento de crianças com fissuras orofaciais nos Estados Unidos.

4. DESENVOLVIMENTO

4.1 ARTIGO I:

Manuscrito submetido para publicação no periódico *Epidemiologic Reviews* (QUALIS CAPES A2) em Jan/2024.

RESEARCH TRENDS IN THE RELATIONSHIP BETWEEN OROFACIAL CLEFT AND CANCER: A BIBLIOMETRIC AND NETWORK VISUALIZATION STUDY

Abstract

The purpose of this study is to use the bibliographic data of publications regarding the association between orofacial cleft and cancer to examine the implications of publication growth, co-citation, co-words, and authorship networks using bibliometric indicators and network visualization. This bibliometric study analyzed documents related to the association between orofacial cleft and cancer published until the present date. Data were obtained in October 2023 from the Elsevier's Scopus, Pubmed, Web of Science. The search strategy was developed, and data obtained were imported into R Studio (package "bibliometrix") for analysis. This bibliometric study found 70 documents published from 1977 to 2023. The majority of publications were journal articles (90.0%) designed as case-control studies (42.8%). American Journal of Epidemiology was the most relevant source. The most global cited documents were Frebourgh T, et al (2006). Vieira A, Martelli-was the top author of the published literature and the most local cited author. Most of the authors was mainly affiliated to Universities from Brazil (University of Montes Claros) and United States (University of Pittsburgh). This bibliometric analysis helps fill research gaps regarding the knowledge of the relationship between orofacial cleft and cancer, providing some clues for selecting future research in this topic.

Key-words: Cleft lip, Cleft palate, Neoplasms, Bibliometrics

Introduction

Orofacial Clefts (OC) and cancer are considered public health problems, contributing to increase morbidity and mortality wordwide[1,2]. Both have a multifactorial etiology, characterized by a complex interaction between genetic and environmental factors, acting either in isolation or in combination[3,4]. These may also have an epigenetic origin, where environmental factors influence gene expression, without necessarily involving genetic changes[5–8]. This multifactorial nature complicates the complete etiological understanding of both pathologies[3,4].

Since both OC and cancer have a multifactorial origin, the hypothesis that these conditions may occasionally have a common etiology[9] is suggested in studies that have found single nucleotide polymorphisms (SNPs) simultaneously associated with both conditions[10–13]. It seems that alterations in genes related to the development of OC have also shown a connection to the carcinogenesis process[8]. Additionally, environmental factors, such as smoking and alcohol consumption, are linked to a higher risk of occurrence for both pathologies[14–16]. Therefore, etiological intersection between OC and cancer could be suggested by environmental factors capable of modulating gene expression in common biological pathways, predisposing to both conditions[8].

Some studies have revealed that individuals with OC and their relatives have an increased risk of developing certain types of cancer such as leukemia, breast cancer, brain cancer, prostate cancer, skin cancer, liver cancer, and colon cancer[9–11,13,17–23]. However, there is no published bibliometric analysis of this topic that offers comprehensive insights into publication frequency, authors, organizations, nations, journals, and references involving cancer across the spectrum of orofacial cleft research.

In that way, the purpose of this study is to use the bibliographic data of publications regarding the association between orofacial cleft and cancer to examine the implications of publication growth, co-citation, co-words and authorship networks using bibliometric indicators and network visualization.

Methods

Bibliometric data collection

This bibliometric study obtained data from documents related to the association between orofacial cleft and cancer published until in October 2023 in the databases Elsevier's Scopus, Pubmed, Web of Science.

The search strategy was developed based on previous work involving bibliometric descriptive analyses and science mappings of the literature[24,25]. For that, the investigation question was defined based on the PECOs (population, exposure, comparison, outcome, study design) (Online Resource 1). The search terms were delected using the Medical Subject Headings (MeSH) and combined using the Boolean operators (Online Resource 2) to create the search strategy used.

After the search, a refinement of the documents was done using the search filters. It was included all publications that had studied orofacial cleft and cancer in association, without refinement filter for years, countries, or languages of studies. The documents found in each databases was exported selecting all necessary fields (abstracts, authors' names, country, article titles, journal names, keywords, citations, and affiliations), in text and BibTex format. These records were then imported into R Studio (package "bibliometrix") for analysis.

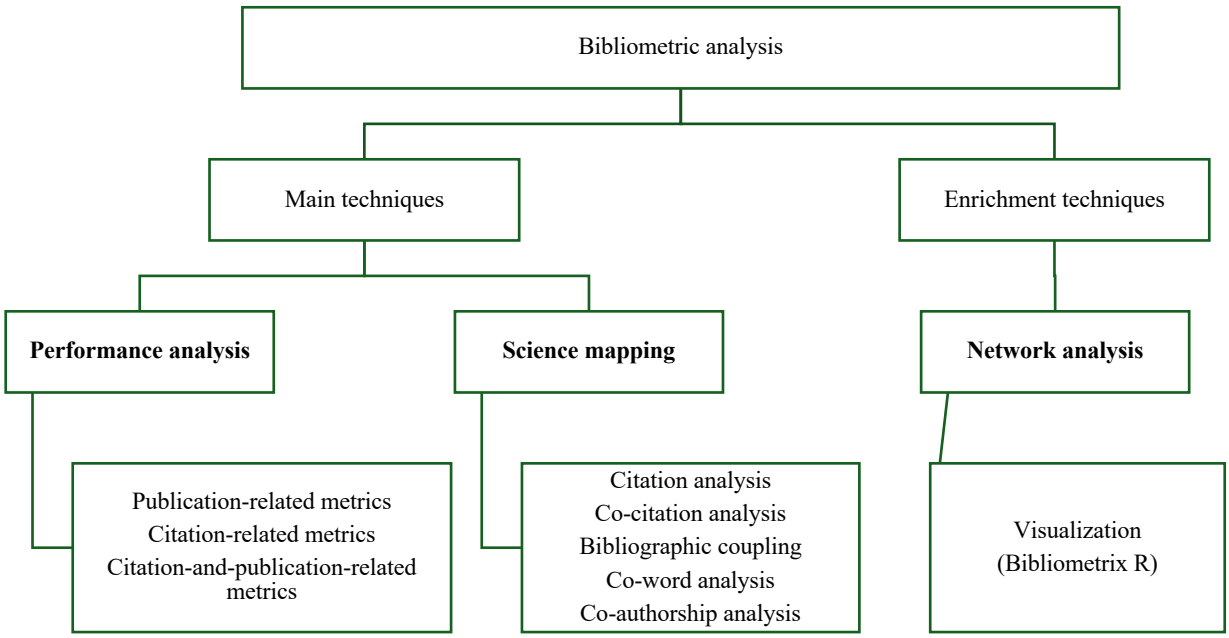
Data Analysis

The data were analyzed using R Studio of Windows, package "bibliometrix"[26]. The data extracted were run through the R program using the commands in the bibliometrix package installed in R[27]. The research constituents (authors, institutions, countries, and journals), document type, annual scientific production on the topic, published sources, keywords and author keywords, author collaboration, most productive authors, author affiliation, author dominance factor, h-index, g-index, m-index, citations, and other essential variables were examined in order to perform the analyzes.

The analyzes included performance analysis (examines research constituents) and science mapping (examines the relationships between research constituents) executed through network visualization tool (Bibliometrix in R) (Figure 1). In the diagrams, nodes indicate different types of information, such as author, and keyword. The nodes of varying sizes represent the

frequency, while the links between each node represent relationships associated with co-citation, co-keyword, or co-authorship.

Figure 1 - Data analysis performed



Font: Donthu N et al., (2021)[25] adapted

Results and discussion

This study examined how much research has been done on the relationship between orofacial cleft and cancer worldwide using a keyword search on the Scopus, Web of Science and PubMed database. The hypothesis that orofacial cleft and cancer may have a common etiology is based in finds that these conditions share genetic and environmental in common[9].

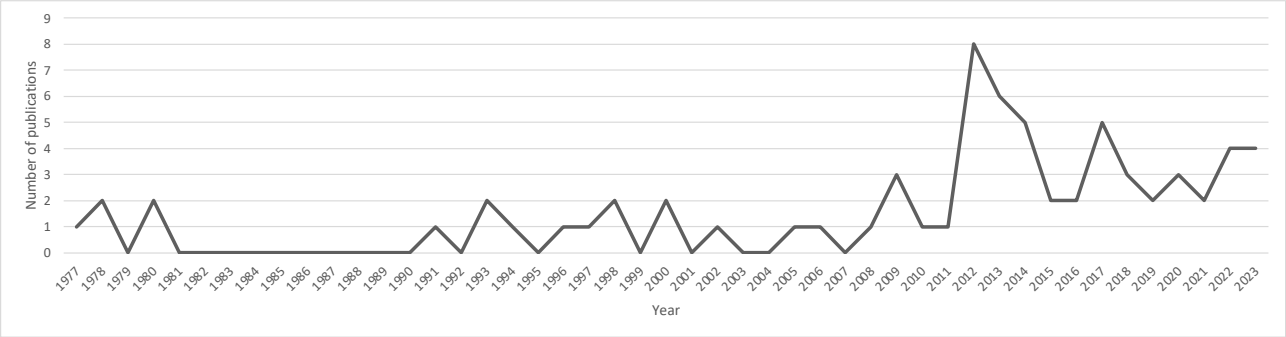
Performance analysis

The results considered 70 documents related to the association between orofacial cleft and cancer. A total of 402 different authors appeared in the search. Single-authored documents were three, co-authors per document were 6.9, and documents with international co-authors were 17.1%. In total, 395 works are cited, with 12.9% average citations per document.

The first publication occurred in 1977 and has continued to date (2023). Annual scientific production showed a peak in 2012 with eight documents. The topic showed an annual growth

rate of 3.1%, indicating that there is renewed interest from the international scientific community in disseminating knowledge in this field of study (Figure 2).

Figure 2 – Annual scientific production

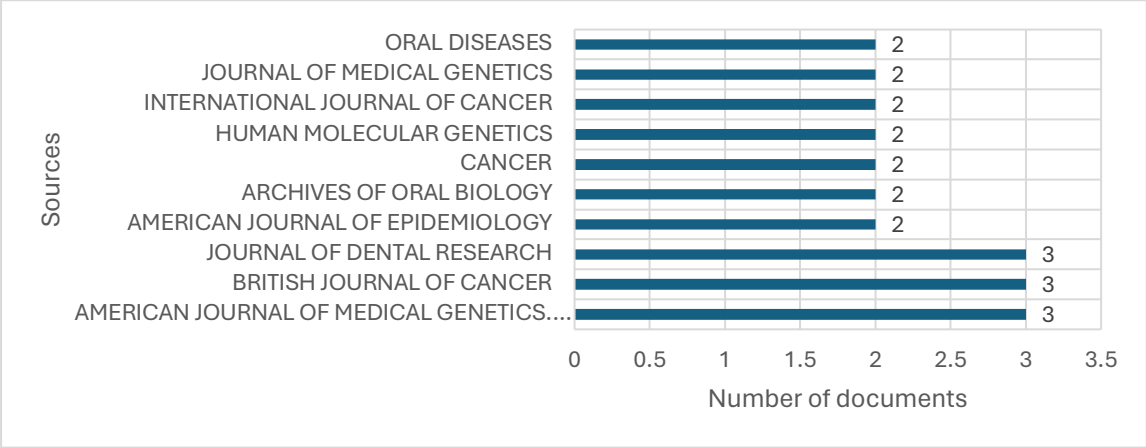


The majority of publications were journal articles (90.0%) designed as case-control studies (42.8%) (Table 1). The American Journal of Medical Genetics, part A (3), British Journal of cancer (3), and Journal of Dental Research (3) were the three top journals that published the literature on this topic (Figure 3).

Table 1 - Various document types in the analyzed data

Document types	n (%)
Book chapter	2
Journal article; not specified	11
Journal article; case control	30
Journal article; case reports	11
Journal article; cohort	11
Letter	5

Figure 3 – The 10 most relevant sources



Citation-publication-related metrics analyze the impact factor of sources and authors using some indexes (h-index, g-index, and m-index). The h-index and the g-index are mainly influenced by the number of publications an author publishes. While the h-index highlights the number of papers with citations number higher or equal to h, the g-index gives more weightage to highly cited articles. Citation-publication-related metrics from the 10 most relevant sources and authors by the h-index are shown in Table 2. American Journal of Epidemiology was the most relevant source, presenting 2 publications cited at least 2 times (h-index = 2), and 2 publications receiving together at least 4 citations (g-index = 2). Among the authors, Vieira A was one of the most relevant, presenting 3 publications cited at least 3 times (h-index = 3), and 7 publications receiving together at least 49 citations (g-index = 7).

Table 2 – Citation-publication-related metrics from the 10 most relevant sources and authors who published in the topic

Element	h_index	g_index	m_index	TC	NP	PY_start
Source						
American Journal of Epidemiology	2	2	0.10526316	127	2	2005
British Journal of Cancer	2	3	0.07692308	13	3	1998
Cancer	2	2	0.06451613	24	2	1993
Human Molecular Genetics	2	2	0.18181818	72	2	2013
Journal of Medical Genetics	2	2	0.04255319	139	2	1977
Plos One	2	2	0.18181818	74	2	2013
Acta Odontologica Scandinavica	1	1	0.1	9	1	2014
Am. J. Hum. Genet	1	1	0.03703704	43	1	1997
Annals Of Plastic Surgery	1	1	0.16666667	12	1	2018
Archives Of Oral Biology	1	2	0.11111111	11	2	2015
Author						
Vieira A	3	7	0.2	56	3	2009
Costa M	3	3	0.27272727	54	3	2013
Figueiredo J	3	4	0.25	106	4	2012
Granjeiro J	3	3	0.27272727	54	3	2013
Seruca R	3	3	0.16666667	218	3	2006
Tannure P	3	3	0.27272727	54	3	2013
Carneiro F	2	2	0.11111111	175	2	2006
Coletta R	2	4	0.2	18	5	2014
Dias V	2	4	0.28571429	19	4	2017
Ho B	2	2	0.18181818	43	2	2013

h-index: h number of publications cited at least h times each one (measure of impact)
g-index: g number of publications receiving combined at least g^2 citations (measure of impact)
m-index: highlights the h-index since the first publication (measure of impact)
total citation (TC), net production (NP), and starting year (PY_start)

Science mapping

Citation analysis represents the relevance of a publication according to the number of citations that it receives, allowing ascertaining the most influential publications in a research field[25]. The most global cited documents analysis revealed that Frebourgh T, et al (2006)[28] was the most influential publication in the field, followed by Bille C et al., (2005)[17] and Zack M et al., (1991)[29]. (Table 3).

Table 3 – The 10 most global cited documents

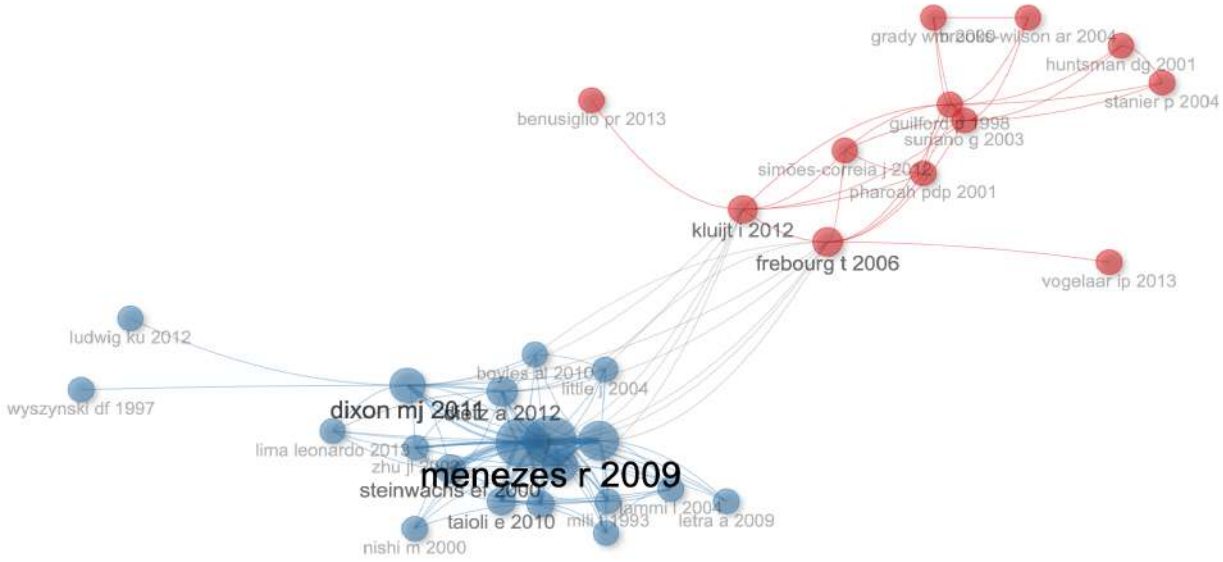
Document	DOI	TC	TC/Year
Frebourg T, 2006, J Med Genet-A	10.1136/jmg.2005.031385	130	7.22
Bille C, 2005, Am J Epidemiol-A	10.1093/aje/kwi132	96	5.06
Zack M, 1991, Cancer Res-A		86	2.61
Botto L, 2013, Plos One	10.1371/journal.pone.0069077	64	5.82
Mertens A C, 1998, The Journal of Pediatrics	doi.org/10.1016/S0022-3476(98)70100-3	63	2.42
Oliveira C, 2013, Prog Mol Biol Transl Sci	10.1016/B978-0-12-394311-8.00015-7	45	4.09
Vogelaar I, 2013, Hum Mol Genet-A	10.1093/hmg/dds497	43	3.90
Narod S A, 1997, Am. J. Hum. Genet		43	1.59
Bjørge T, 2008, Cancer Epidemiol Biomarkers Prev	10.1158/1055-9965.EPI-07-2630	41	2.56
Kuechler E, 2013, J Dent Res	10.1177/0022034512468750	34	3.09

Total citation (TC); Total citation per year (TC/Year)

We also completed bibliographic coupling, co-citation, co-word, and co-authorship analyses. In the co-occurrence diagrams, circle size indicates the number of publications that match the relevant aspect (i.e. document, word, or author), and the distance between two nodes and the width of the line connecting them indicate the degree to which the aspect co-occurs. The closer two nodes are situated, and the thicker the line connecting them are, more likely there is to have a strong connection between them.

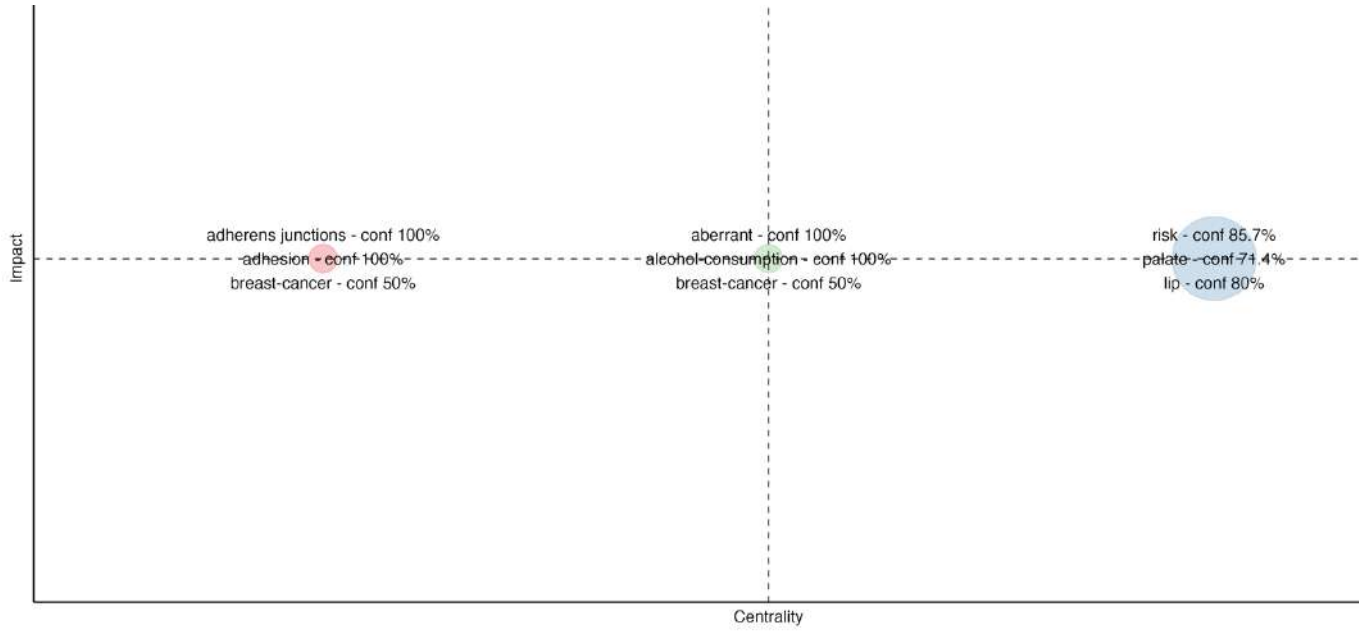
Co-citation of two articles occurs when both are cited in a third article[26]. Assuming that publications cited together frequently are similar thematically[25], co-citation analysis allows to understand the development of the foundational themes in a research field. Here, there were two clusters of cited-reference works (nodes). According with their circle size and strength in the diagram, Menezes R at al., (2009)[12], in the blue cluster, and Frebough T et al., (2006)[28] and Kluijit I et al., (2012)[30], in the red cluster, seem to have a greater likelihood to be cited with other documents in a single article. (Figure 4).

Figure 4 – Co-citation network analysis



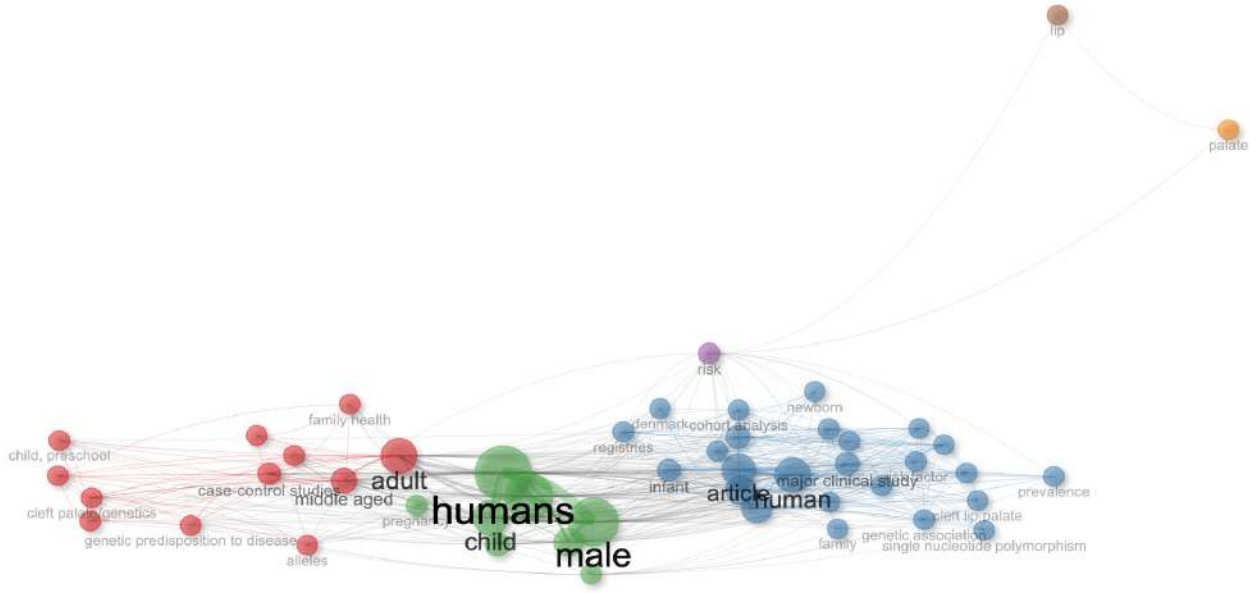
Two articles are bibliographically coupled if at least one cited source appears in the reference lists of both articles[26]. It implies that two publications sharing common references are also similar in their content[25,26]. In that way, bibliographic coupling analyzes the present relationships among citing publications to understand the development of themes in a research field[25]. This analysis identified three thematic clusters of publications based on shared references, uncovering a broad spectrum of themes and its latest developments (Figure 5).

Figure 5 - Bibliographic coupling analysis



The co-word analysis explores future relationships among topics in a research field by focusing on the written content (words) of the publication itself[25]. In order to do that, it draws the conceptual structure of a framework using a word co-occurrence network to map or cluster terms extracted from the keywords, titles, or abstracts[26]. The co-word diagram identified three major clusters, and the words that were consistently at the center position of each one included "adult," "human," and "human," respectively (Figure 6). Other three minor clusters included "risk", "lip", and "palate" in the center position. Words with betweenness centrality greater than 10 included as "cleft lip," "cleft palate," "article," "male," "female," and "child,". While words with betweenness centrality greater than 5 included as "middle age," "neoplasms," "adolescent," and "risk factors". From that, it can be implied that mostly research regarding orofacial cleft and cancer has been done on humans, males and females were studied equally, and mainly studies were related to child and adult. Similar findings were reported in the study of orofacial clefts[31]. Some of the least co-occurred words in this research field are "pregnancy", "newborn", "prevalence" and "family health".

Figure 6 - Word co-occurrences analysis



Regarding the type of cancer analyzed in association with orofacial cleft, the most specified types were gastric cancer, leukemia, breast cancer, childhood cancer, oral cancer, nevoid basal cell carcinoma and thyroid cancer (Table 4).

Table 4 - Various types of cancer studied in association with orofacial cleft

Cancer type	n
Cancer (non-specified)	35
Gastric cancer	7
Leukemia	5
Breast cancer	4
Childhood cancer	4
Nevoid basal cell carcinoma	3
Oral cancer	2
Thyroid cancer	2
The basal cell nevus syndrome	1
Brain tumors	1
Minor salivary gland	1
Colorectal cancer	1
Musculo aponeurotic fibrosarcoma	1
Oropharyngeal cancer	1
Osteolipoma	1
Prostate cancer	1
Uterine cancer	1

Vieira A, Martelli-Júnior H, and Martelli D were the top authors of the published literature, with 13, eight and seven publications respectively. Vieira A was also the most local cited author (12 documents) (Figure 7), and the author with more productions over the time (Figure 8). All author local impact metrics were shown in Table 2.

Figure 7 – The 10 most relevant authors and the 10 most cited authors

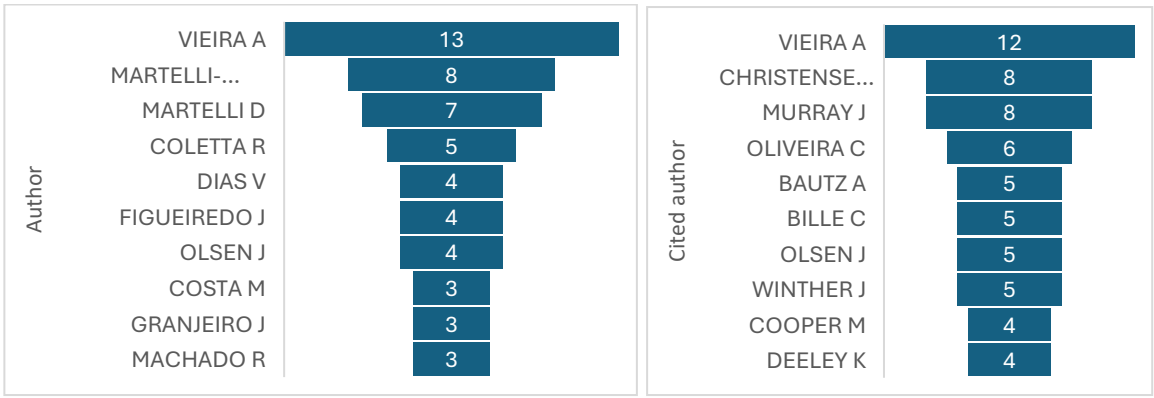
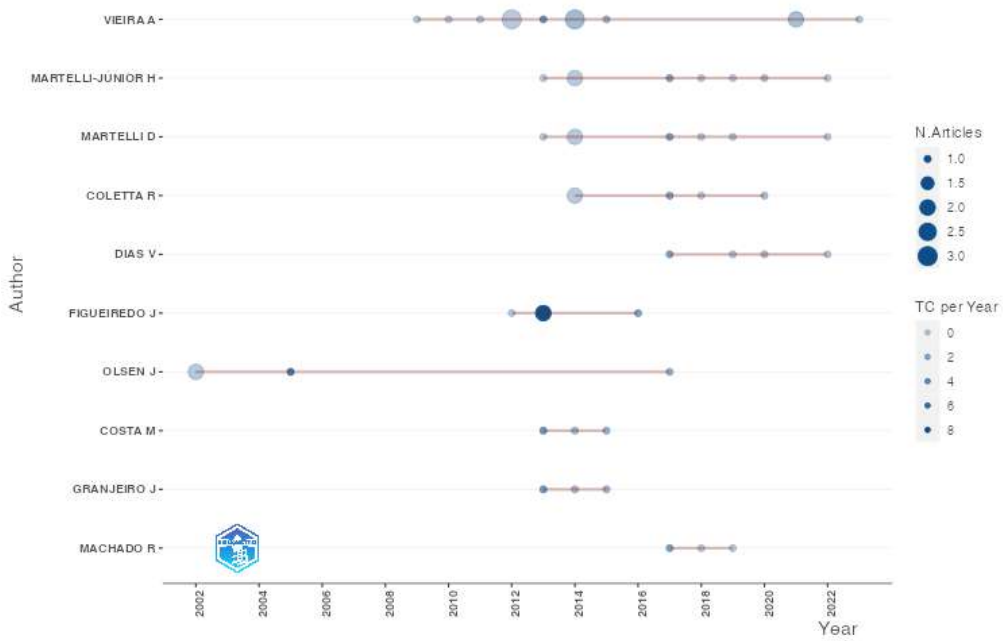


Figure 8 – Top 10 Author's production over the time



Moreover, most of the authors who study about a relationship between orofacial cleft and cancer is mainly affiliated to Universities from Brazil (University of Montes Claros, 25 documents) and United States (University of Pittsburgh) (Figure 9), which are the two countries found with the highest number of publications (Figure 10). These results contribute with a previous findings showing that United States' leads the first position in the study of orofacial cleft[32,33], with significant contributions from important institutions such as the University of Pittsburgh and University of Iowa[32]. This same previous finding demonstrated an increased number of publications about orofacial cleft in Brazil and Netherlands (Radboud University Nijmegen)[32].

Figure 9 – The 10 most relevant affiliations

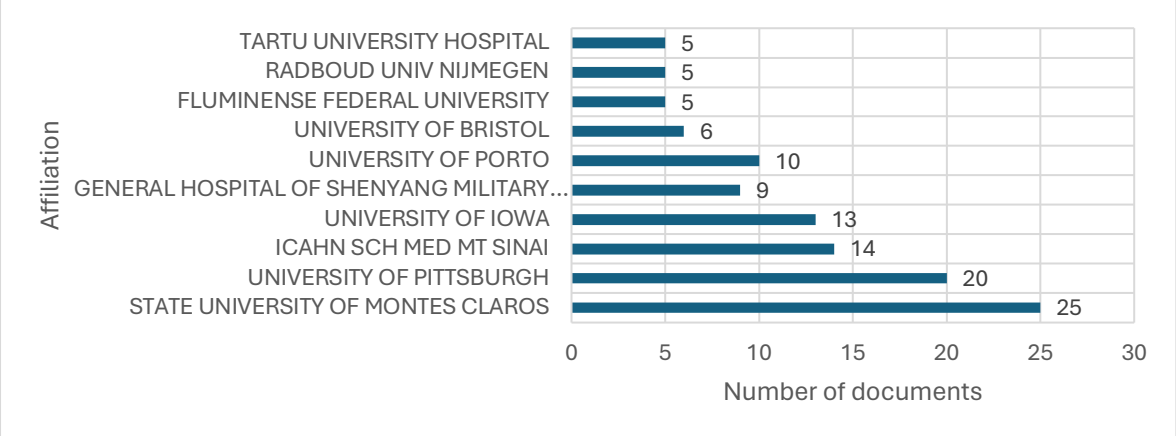
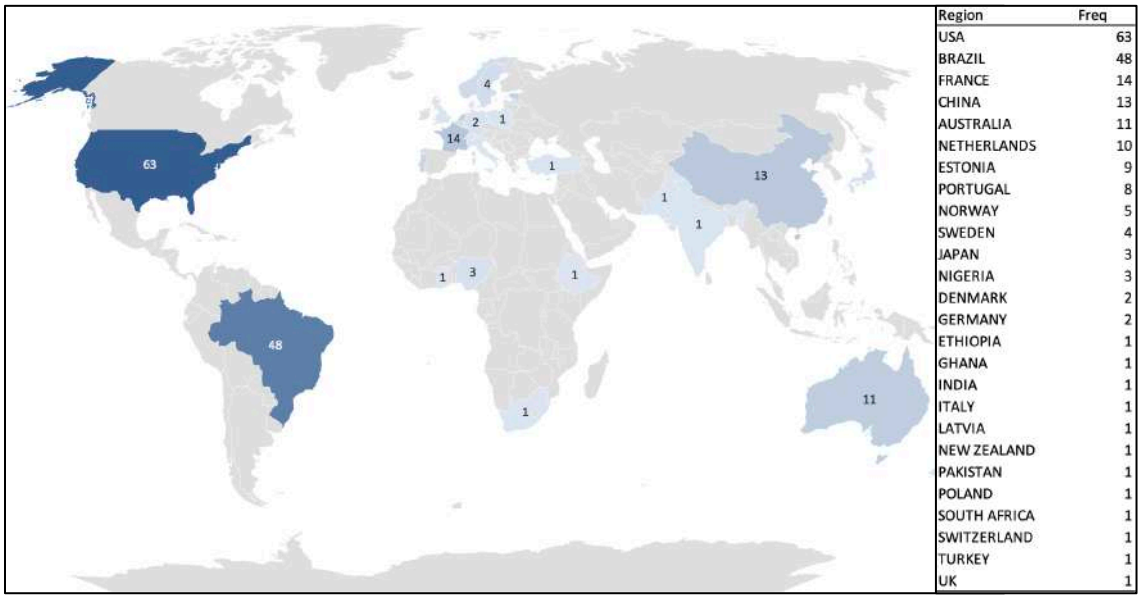
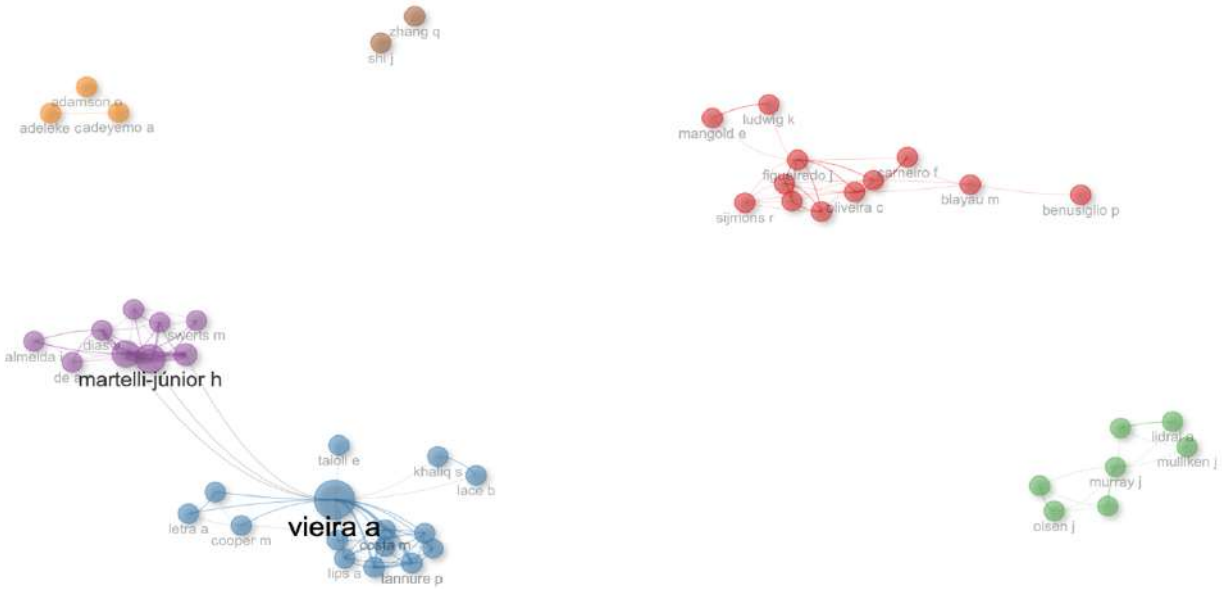


Figure 10 – Country scientific production



Co-authorship analysis uses authors' affiliations (institutions and country) to examine the social interactions or relationships among authors and their affiliations, and equivalent impacts on the development of the research field. The co-authorship analysis revealed six different clusters. The purple and blue authorship clusters showed to interact themselves, highlighting interaction contributions between Vieira A (University of Pittsburgh, United States) and Martelli-Junior H (State University of Montes Claros, Brazil) in the publications in this field (Figure 11).

Figure 11 – Author's collaboration network



There are some limitations in this study that must be considered. First, the literature downloaded may not include all published literature, so it may affect the results of the analysis. However, in order to minimize it and to ensure optimal coverage of the required information, we selected the most commonly used and acceptable database for bibliometric research[34] and combined different MeSH terms in the search strategy. Second, high-frequency co-word analysis is based on the intercept frequency, causing that very low-frequency keywords may be ignored[31,32]. Third, in most of the analysis, this study only showed the top 10 contributions in this field, but the contribution from other authors is also appreciable and creditable. For last, bibliometric searches include the related terms and might not produce specific results but show the overall progress, production, and trend on the topic of interest.

This is the first time that bibliometric analyze methods (citation, co-citation, bibliographic coupling, co-word, and co-authorship) have been used to investigate the fundamental knowledge of the relationship between orofacial cleft and cancer. The current study also provides valuable insights into research constituents (authors, institutions, countries, and journals), with exceptional bibliometric profiles that have made outstanding contributions to the field, as well as their patterns of interaction. Since both orofacial clefts and cancer are public health concerns worldwide[35–38], the present study makes a unique contribution by analyzing the published documents on the topic of cancer in across the full spectrum of orofacial cleft research. Researchers with an interest in this field can therefore take advantage of these results for potential collaboration with the view to producing high-quality research.

Conclusion

Orofacial clefts and cancer are public health concerns worldwide and bibliometric analysis such that can help fill research gaps regarding the knowledge of the relationship between orofacial cleft and cancer. This bibliometric study found 70 documents related to the association between orofacial cleft and cancer published from 1977 to 2023. These studies were analyzed by citation, co-citation, bibliographic coupling, co-word, and co-authorship analysis techniques, providing some clues for selecting future research in this topic.

References

1 Sung H, Ferlay J, Siegel R, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* 2021; **71**: 209–49.

2 Mossey PA, Little J, Munger RG, Dixon MJ, Shaw WC. Cleft lip and palate. *The Lancet* 2009; **374**: 1773–85.

3 Dixon MJ, Marazita ML, Beaty TH, Murray JC. Cleft lip and palate: synthesizing genetic and environmental influences. *NIH Public Access* 2011; **3**: 203.

4 Vieira AR. Basis of Inheritance in Humans. *Monogr Oral Sci* 2022; 30: 1–19.

5 Leslie EJ. Genetic models and approaches to study orofacial clefts. *Oral Dis* 2022; **28**: 1327–38.

6 Fox SA, Tiwari L, Farah CS. *Epigenetics and oral disease*. Elsevier Inc., 2019. https://doi.org/10.1016/B978-0-12-813762-8.00007-4.

7 Mohammed SI, Springfield S, Das R. Role of epigenetics in cancer health disparities. *Methods in Molecular Biology* 2012; **863**: 395–410.

8 Cruz LA. *Mecanismos genéticos e epigenéticos na etiologia das fissuras orofaciais*. 2017.

9 Vieira AR. Cleft Lip/Palate and Cancer: a True Connection. *Revista Brasileira de Cancerologia* 2021; **67**: 11–3.

10 Vieira AR, Khaliq S, Lace B. Risk of cancer in relatives of children born with isolated cleft lip and palate. *Am J Med Genet A* 2012; **158 A**: 1503–4.

11 Bui AH, Ayub A, Ahmed MK, Taioli E, Taub PJ. Association Between Cleft Lip and/or Cleft Palate and Family History of Cancer. *Ann Plast Surg* 2018; **80**: S178–81.

12 Menezes R, Marazita ML, Mchenry TG, et al. AXIN2, Orofacial Clefts and Positive Family History for Cancer. *J Am Dent Assoc* 2009; **140**: 80–4.

13 Taioli E, Ragin C, Robertson L, Linkov F, Thurman NE, Vieira AR. Cleft lip and palate in family members of cancer survivors. *NIH Public Access* 2010; **28**: 958–62.

14 Martelli DRB, Coletta RD, Oliveira EA, et al. Association between maternal smoking, gender, and cleft lip and palate. *Braz J Otorhinolaryngol* 2015; **81**: 514–9.

15 Lacerda RHW, Furtado PGC, Vieira AR. Maternal Smoking Leads to Larger Cleft Palate Defects. *Frontiers in Dental Medicine* 2021; 1. https://doi.org/10.3389/fdmed.2020.632037.

16 Yang L, Wang H, Yang L, et al. Maternal cigarette smoking before or during pregnancy increases the risk of birth congenital anomalies: a population-based retrospective cohort

study of 12 million mother-infant pairs. *BMC Med* 2022; **20**. https://doi.org/10.1186/s12916-021-02196-x.

17 Bille C, Winther JF, Bautz A, Murray JC, Olsen J, Christensen K. Cancer risk in persons with oral cleft - A population-based study of 8,093 cases. *Am J Epidemiol* 2005; **161**: 1047–55.

18 Dietz A, Pedersen DA, Jacobsen R, Wehby GL, Murray JC, Christensen K. Risk of breast cancer in families with cleft lip and palate. *Ann Epidemiol* 2012; 22: 37–42.

19 Gonçalves E, Martelli DRB, Coletta R Della, Vieira AR, Caldeira AP, Martelli-Júnior H. Risk of leukemia in first degree relatives of patients with nonsyndromic cleft lip and palate. *Braz Oral Res* 2014; **28**: 1–3.

20 Machado RA, Popoff DAV, Martelli-Júnior H. Relationship between non-syndromic oral clefts and cancer: a systematic review and meta-analysis. *Oral Dis* 2022. https://doi.org/10.1111/odi.14179.

21 Letra A, Menezes R, Granjeiro JM, Vieira AR. AXIN2 and CDH1 polymorphisms, tooth agenesis, and oral clefts. *Birth Defects Res A Clin Mol Teratol* 2009; 85: 169–73.

22 Yildirim M, Seymen F, Deeley K, Cooper ME, Vieira AR. Defining predictors of cleft lip and palate risk. *J Dent Res* 2012; **91**: 556–61.

23 Zhu JL, Basso O, Hasle H, Winther JF, Olsen JH, Olsen JH. Do parents of children with congenital malformations have a higher cancer risk? A nationwide study in Denmark. *Br J Cancer* 2002; **87**: 524–8.

24 Wu T, Duan Y, Zhang T, Tian W, Liu H, Deng Y. Research Trends in the Application of Artificial Intelligence in Oncology: A Bibliometric and Network Visualization Study. *Frontiers in Bioscience* - Landmark 2022; 27. https://doi.org/10.31083/j.fbl2709254.

25 Donthu N, Kumar S, Mukherjee D, Pandey N, Lim WM. How to conduct a bibliometric analysis: An overview and guidelines. *J Bus Res* 2021; **133**: 285–96.

26 Aria M, Cuccurullo C. bibliometrix: An R-tool for comprehensive science mapping analysis. *J Informetr* 2017; **11**: 959–75.

27 No authors listed: Bibliometrix command in R Program. Bibliometrix - Home 2023. https://www.bibliometrix.org/home/ (accessed 11 Oct 2023).

28 Frebourg T, Oliveira C, Hochain P, et al. Cleft lip/palate and CDH1/E-cadherin mutations in families with hereditary diffuse gastric cancer. *J Med Genet* 2006; **43**: 138–42.

29 Zack M, Adami H-O, Ericson A. Maternal and Perinatal Risk Factors for Childhood Leukemia. *Cancer Res* 1991; **51**: 3696–701.

30 Kluijt I, Siemerink EJM, Ausems MGEM, et al. CDH1-related hereditary diffuse gastric cancer syndrome: Clinical variations and implications for counseling. *Int J Cancer* 2012; **131**: 367–76.

31 Jadhav V V., Kale B, Meshram MG. Bibliometric Analysis of Publications on Cleft Lip and Palate. *ECS Trans* 2022; **107**: 18447–56.

32 Zhang Q, Yue Y, Shi B, Yuan Z. A Bibliometric Analysis of Cleft Lip and Palate-Related Publication Trends From 2000 to 2017. *Cleft Palate-Craniofacial Journal* 2019; **56**: 658–69.

33 Lei K, Wang X, Liu Y, Sun T, Xie W. Global research hotspots and trends of the Notch signaling pathway in the field of cancer: a bibliometric study. *Am J Trans Res* 2022; **14**: 4918–30.

34 Yeung AWK. Comparison between Scopus, Web of Science, PubMed and publishers for mislabelled review papers. *Curr Sci* 2019; **116**: 1909–14.

35 Agbenorku P. Orofacial Clefts: A Worldwide Review of the Problem. *ISRN Plastic Surgery* 2013; 1–7.

36 Denadai R, Lo LJ. The Burden of Orofacial Clefts: Global Trends and Focus on Underresourced Regions. Current Concept in Cleft Surgery. Singapore.: Springer, 2022.

37 World Cancer Research Fund International. Worldwide cancer data. 2020.

38 Malicka I, Hojan K, Hanuszkiewicz J. Cancer as a Public Health Problem--Prevention of Cancer. *Int J Environ Res Public Health* 2021.

Online Resource

Online Resource 1 - PECOs question strategy (Patient, Exposition, Comparison, Outcome, and Study Design)

PECO	Description	MESH and entry terms selected
P	Individuals with cancer	**Neoplasms** Entry Terms: Tum Neoplasm Tums Neoplasia Neoplasias Cancer Cancers Malignant Neoplasm Malignancy Malignancies Malignant Neoplasms Neoplasm, Malignant Neoplasms, Malignant
		AND
E	Presence of orofacial cleft	**orofacial Cleft** Entry Terms: Cleft Lip-Palate, Nonsyndromic Cleft Lip with without Cleft Palate, Nonsyndromic orofacial Cleft, Nonsyndromic OFC **Cleft Lip** Entry Terms: Cleft Lips Lip, Cleft Lips, Cleft Harelip Harelips **Cleft Palate** Entry Terms: Cleft Palates Palate, Cleft Palates, Cleft Cleft Palate, Isolated
C	Absence of orofacial cleft	No descriptors included
O	frequency of cancer orofacial cleft	No descriptors included
		AND NOT
S	Observational studies (case reports, case–-control studies, and cohort studies)	**Review** Entry Terms: Review, Multicase Review, Academic Review of Reported Cases Review Literature **Systematic review** Entry Terms: Review, Systematic Meta analysis

Online Resource 2 – String search strategy used in each database

Database	String search strategy	n (date)

Pubmed	((((((((((((((Cancer[Title/Abstract]) (Tum[MeSH Terms])) (Neoplasm[MeSH Terms])) (Tums[MeSH Terms])) (Neoplasia[MeSH Terms])) (Neoplasias[MeSH Terms])) (Cancer[MeSH Terms])) (Cancers[MeSH Terms])) (Malignant Neoplasm[MeSH Terms])) (Malignancy[MeSH Terms])) (Malignancies[MeSH Terms])) (Malignant Neoplasms[MeSH Terms])) (Neoplasm, Malignant[MeSH Terms])) (Neoplasms, Malignant[MeSH Terms])) OR (Neoplasms[MeSH Terms])) AND ((((((((((((((("orofacial Cleft"[Title/Abstract]) ("Cleft Lip-Palate, Nonsyndromic"[MeSH Terms])) ("Cleft Lip with without Cleft Palate, Nonsyndromic"[MeSH Terms])) ("orofacial Cleft, Nonsyndromic"[MeSH Terms])) (OFC[MeSH Terms])) ("Cleft Lip"[Title/Abstract])) ("Cleft Lips"[MeSH Terms])) ("Lip, Cleft"[MeSH Terms])) ("Lips, Cleft"[MeSH Terms])) (Harelip[MeSH Terms])) (Harelips[MeSH Terms])) ("Cleft Palate"[Title/Abstract])) ("Cleft Palates"[MeSH Terms])) ("Palate, Cleft"[MeSH Terms])) ("Palates, Cleft"[MeSH Terms])) ("Cleft Palate, Isolated"[MeSH Terms])) OR (Cleft Palate[MeSH Terms]))) NOT (((((((((Review[Title/Abstract]) OR (Review[MeSH Terms])) OR (review, academic[MeSH Terms])) OR (review of reported cases[MeSH Terms])) OR (review literature[MeSH Terms])) OR (Systematic review[Title/Abstract])) OR (review, systematic[MeSH Terms])) OR (meta analysis[MeSH Terms])) OR (meta analysis [Title/Abstract]))	560 (18/10/23)
Web of Science	((((((((((((TS=(Neoplasms)) OR TS=(Tum)) OR TS=(Neoplasm)) OR TS=(Tums)) OR TS=(Neoplasia)) OR TS=(Neoplasias)) OR TS=(Cancer)) OR TS=(Cancers)) OR TS=(Malignant Neoplasm)) OR TS=(Malignancy)) OR TS=(Malignancies)) OR TS=(Malignant Neoplasms)) OR TS=(Neoplasm, Malignant)) OR TS=(Neoplasms, Malignant) AND ((((((((((((((TS=(orofacial Cleft)) OR TS=(Cleft Lip-Palate, Nonsyndromic)) OR TS=(Cleft Lip with without Cleft Palate, Nonsyndromic)) OR TS=(Orofacial Cleft, Nonsyndromic)) OR TS=(OFC)) OR TS=(Cleft Lip)) OR TS=(Cleft Lips)) OR TS=(Lip, Cleft)) OR TS=(Lips, Cleft)) OR TS=(Harelip)) OR TS=(Harelips)) OR TS=(Cleft Palate)) OR TS=(Cleft Palates)) OR TS=(Palate, Cleft)) OR TS=(Palates, Cleft)) OR TS=(Cleft Palate, Isolate) NOT (((((((TS=(Review)) OR TS=(Review, Multicase)) AND TS=(Review, Academic)) OR TS=(Review of Reported Cases)) OR TS=(Review Literature)) OR TS=(Systematic review)) OR TS=(Review, Systematic)) OR TS=(Meta analysis)	563 (18/10/23)
Scopus	(TITLE-ABS-KEY (neoplasms) OR TITLE-ABS-KEY (tum) OR TITLE-ABS-KEY (neoplasm) OR TITLE-ABS-KEY (tums) OR TITLE-ABS-KEY (neoplasia) OR TITLE-ABS-KEY (neoplasias) OR TITLE-ABS-KEY (cancer) OR TITLE-ABS-KEY (cancers) OR TITLE-ABS-KEY (malignant AND neoplasm) OR TITLE-ABS-KEY (malignancy) OR TITLE-ABS-KEY (malignancies) OR TITLE-ABS-KEY (malignant AND neoplasms) OR TITLE-ABS-KEY (neoplasm, AND malignant) OR TITLE-ABS-KEY (neoplasms, AND malignant)) AND (TITLE-ABS-KEY (orofacial AND cleft) OR TITLE-ABS-KEY (cleft AND lip-palate, AND nonsyndromic) OR TITLE-ABS-KEY (cleft AND lip AND with AND without AND cleft AND palate, AND nonsyndromic) OR TITLE-ABS-KEY (orofacial AND cleft, AND nonsyndromic) OR TITLE-ABS-KEY (ofc) OR TITLE-ABS-KEY (cleft AND lip) OR TITLE-ABS-KEY (cleft AND lips) OR TITLE-ABS-KEY (lip, AND cleft) OR TITLE-ABS-KEY (lips, AND cleft) OR TITLE-ABS-KEY (harelip) OR TITLE-ABS-KEY (harelips) OR TITLE-ABS-KEY (cleft AND palate) OR TITLE-ABS-KEY (cleft AND palates) OR TITLE-ABS-KEY (palate, AND cleft) OR TITLE-ABS-KEY (palates, AND cleft) OR TITLE-ABS-KEY (cleft AND palate, AND isolate)) AND NOT (TITLE-ABS-KEY (review) OR TITLE-ABS-KEY (review, AND academic) OR TITLE-ABS-KEY (review AND of AND reported AND cases) OR TITLE-ABS-KEY (review AND literature) OR TITLE-ABS-KEY (systematic AND review) OR TITLE-ABS-KEY (review, AND systematic) OR TITLE-ABS-KEY (meta AND analysis))	1,107 (18/10/23)

ORIGINAL ARTICLE

https://doi.org/10.1590/1984-0462/2024/42/2022234

Epidemiologic profile and prevalence of live births with orofacial cleft in Brazil: a descriptive study

Perfil epidemiológico e prevalência de nascidos vivos com fissura orofacial no Brasil: um estudo descritivo

Adriana Mendonça da Silva[a], Rodrigo Tripodi Calumby[a], Valéria Souza Freitas[a,*]

ABSTRACT

Objective: To describe the epidemiological profile and prevalence of live births with orofacial clefts in Brazil between 1999 and 2020.

Methods: Descriptive study. The population corresponded to live births with isolated orofacial clefts in Brazil registered in the Live Birth Information System between 1999 and 2020. Descriptive variables were selected according to their availability and grouped into socioeconomic and demographic, maternal and child health care, and biological variables. Data were submitted to a descriptive analysis using the Software for Statistics and Data Science (STATA).

Results: During the period, 33,699 children were born with orofacial clefts, and 82.1% (27,677) of them were isolated clefts. Regarding these cases, the majority were cleft lip and palate (9,619 or 34.7%), followed by cleft palate (9,442 or 34.1%), and by cleft lip (8,616 or 31.3%).

Conclusions: Live births with orofacial clefts in Brazil were male, white, with birthweight ≥2,500 g and gestational age ≥37 weeks, born by cesarean section, and with Apgar scores ≥7. The cases were more frequent among mothers who were in their first and single pregnancy and had seven or more prenatal appointments. The mothers were 20 and 29 years old, had eight to ten years of study, and were single. The national prevalence of clefts was 4.24/10,000. The South and Southeast regions of Brazil had the highest prevalence, while the lowest prevalence was recorded in the Northeast and North regions. For the Federative Units, the highest and lowest prevalences were found, respectively, in Paraná and Acre.

Keywords: Epidemiology; Cleft palate; Cleft lip; Congenital abnormalities.

RESUMO

Objetivo: Descrever o perfil epidemiológico e a prevalência dos nascidos vivos com fissuras orofaciais no Brasil entre 1999 e 2020.

Métodos: Estudo descritivo. A população correspondeu aos nascidos vivos com fissuras orofaciais isoladas no Brasil registrados no Sistema de Informação de Nascidos Vivos entre 1999 e 2020. As variáveis descritivas foram selecionadas de acordo com a sua disponibilidade e agrupadas em variáveis socioeconômicas e demográficas, de atenção à saúde materno-infantil e biológicas. Os dados foram submetidos a análise descritiva utilizando o *Software for Statistics and Data Science* (STATA).

Resultados: No período, 33.699 indivíduos nasceram com fissura orofacial no Brasil, e 82,1% (27.677) deles foram fissuras isoladas. Com relação a esses casos, a maioria foi de fissuras de lábio e palato (9.619 ou 34,7%), seguidas por fissura de palato (9.442 ou 34,1%) e por fissura de lábio (8.616 ou 31,1%).

Conclusões: O perfil epidemiológico dos nascidos vivos com fissuras orofaciais no Brasil foi de nascidos do sexo masculino, da raça/cor branca, por parto cesáreo, com peso ao nascer ≥2,500 g, idade gestacional ≥37 semanas e com índices de Apgar ≥7. Os casos foram mais frequentes entre mães que estavam na primeira gestação, única e que haviam realizado sete ou mais consultas de pré-natal. As mães, com maior frequência, tinham entre 20 e 29 anos, apresentavam oito ou mais anos de estudo, eram solteiras e residiam em cidades do interior. A prevalência nacional de fissuras foi de 4,24/10.000. As Regiões Sul e Sudeste apresentaram as maiores prevalências, enquanto as menores foram registradas nas Regiões Nordeste e Norte. Para as Unidades Federativas, as maiores e menores prevalências foram encontradas, respectivamente, no Paraná e no Acre.

Palavras-chave: Epidemiologia; Fissura palatina; Fenda labial; Anormalidades congênitas.

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INTRODUCTION

Orofacial clefts (OC) are the most common craniofacial congenital malformation and occur due to a failure in the embryological fusion process.[1] According to their embryological origin, clefs can be divided as cleft lip with or without palate (CL/P) and cleft palate only (CP). Also, they can be classified based on the presence or absence of any other anomaly such as syndromic (SOC) or non-syndromic (NSOC) clefts, respectively.[2] The latter can also be named as isolated clefs, since there are no other anomalies or concomitant syndromes.

Worldwide, it is estimated that they affect approximately 1.5 to 1,000 births, which corresponds to about 220,000 new cases per year.[3] However, this rate can vary greatly from country to country. The highest prevalences were found in Japan (20/10,000), Canada (10.5/10,000), the United States (10.2/10,000) and Australia (9.7/10,000).[4] In low- and middle-income countries, approximately one in every 730 children are born with OC.[5] In Brazil, few studies have been carried out on the national prevalence of OC, mainly due to reporting and recording difficulties.[6] The most recent studies[7,8] found prevalences near 5.1/10,000 live births for the country.

The impacts caused by OC are related to aesthetic, functional and emotional alterations, which can last for the entire life of the individual — such as facial disfigurement, recurrent infections, social stigma, and speech, hearing and teeth formation disabilities.[1] In addition, OC represent one of the main causes of morbidity in the world.[1] Despite the many advances in OC treatment options, this continues to be a serious burden worldwide.[9] The prevalence of OC, together with the need for a long-term multidisciplinary treatment and the economic impact generated by them, has led the World Health Organization to consider them as a public health problem.[10]

The etiology of OC is considered complex and multifactorial, involving the interaction of genetic, environmental and behavioral factors.[2] Despite these, socioeconomic inequalities may also be related to OC.[11] A maternal profile of adverse risk, involving less favored social strata and less accessibility to the health system, indicates that such mothers have more difficulty in accessing prenatal care services, which may favor increased morbidity, infant mortality and delayed diagnoses of these malformations.[12]

Therefore, knowledge of factors such as education, family income status and stressful events during pregnancy can provide clues about the inequalities that need to be addressed by health professionals in order to prevent and control the identified risk factors associated with the occurrence of OC, starting with the application of specific measures to promote health during pregnancy.[13] For this, epidemiological studies that assess the socioeconomic, cultural and environmental conditions of patients with OC are needed.[5,6] In Brazil, these studies are mostly locally specific, as they propose to describe this event using local data from a specific city, state or region.[14-16] Thus, it is essential to study population data in a national context, not only to derive situational knowledge on the OC problem in Brazil, but also to aid the planning of public policies for assistance and prevention.[17] Therefore, the present study aimed to describe the epidemiological profile and prevalence of live births with isolated OC in Brazil between 1999 and 2020.

METHOD

This is an observational, descriptive study, conducted using data collected in the Live Birth Information System (SINASC) about children born with OC in Brazil between 1999 and 2020. SINASC is a national open access health information system that makes it possible to monitor the population's health situation through the collection and processing of demographic and epidemiological data on newborns, mothers, prenatal care and delivery.

The study population corresponded to all cases of live births in Brazil registered in SINASC as having isolated OC (no record of other anomalies or concomitant syndromes) between 1999 and 2020. The OC classification adopted by SINASC follows the Tenth Revision of the International Statistical Classification of Diseases and Related Health Problems (CID-10), which classifies clefts into the following groups: Q35 (cleft palate — CP), Q36 (cleft lip — CL) and Q37 (cleft lip and palate — CLP). Live births that were coded in SINASC for CL and CP simultaneously were considered as having CLP. Cases of OC registered in association with any other major or minor defects, syndromes or multiple birth defects were excluded. Likewise, cases recorded as cleft uvula, or atypical or oblique cleft were also excluded.[4]

The selection of descriptive variables was carried out taking into account their availability in the database. After that, the variables were grouped into socioeconomic and demographic, maternal and child health care and biological variables (Table 1). The quantitative variables (maternal age, paternal age, number of previous pregnancies, Apgar 1st and 5th minute and birth weight in grams) were categorized according to the classification of the Brazilian Ministry of Health.[14]

Data were submitted to statistical analysis using the Software for Statistics and Data Science (STATA), version 10. Descriptive analysis was performed according to socioeconomic, maternal and child care and biological variables in absolute and relative frequencies. Differences between groups were tested using the chi-square test, establishing a significance level of $p<0.05$. Data were also analyzed in a bivariate way to show the crude

association between the sex and the NSOC types occurrence. The prevalence of OC was calculated at the national, regional and federal levels. For this calculation, the number of live births with OC according to the mother's state of residence was divided by the total number of live births in the same year and place and multiplied by 10,000.

Table 1. Description and categorization of the selected descriptive variables.

Variables	Description	Categorization
Biological variables		
Orofacial cleft type	According to CID-10	Cleft lip; Cleft palate; Cleft lip with palate.
Sex of newborn	Biological sex of newborn	Male; Female.
Ethnicity of newborn	Color of newborn as declared by the mother	White; Black; Yellow (Asian); Brown; Indigenous.
Maternal age	Number of complete years of mother at the time of delivery	≤19 years old; 20–29 years old; 30–34 years old; 35–39 years old; ≥40 years old.
Paternal age	Number of complete years of newborn's father	≤19 years old; 20–39 years old; ≥40 years old.
Apgar score 1st minute	Assess newborn's general condition and vitality in the first minute. It is a predictor of the infant's chances of surviving the first year of life. It ranges from 0 to 10. A score of 7 or greater indicates that the neonate is in good to excellent physical condition.	<7; ≥7 (satisfactory).
Apgar score 5th minute	Assess newborn's general condition and vitality in the first 5 minutes. It is a predictor of the infant's chances of surviving the first year of life. It ranges from 0 to 10. A score of 7 or greater indicates that the neonate is in good to excellent physical condition.	<7; ≥7 (satisfactory).
Maternal and child health care variables		
Number of previous pregnancies	Number of previous pregnancies, not including current pregnancy.	None; One; Two or more.
Number of prenatal appointments	Number of prenatal appointments.	None; 1 to 3 appointments; 4 to 6 appointments; 7 or more appointments.
Place of delivery	Place where the birth took place.	Home; Hospital; Other health place; Others.
Type of delivery	How the birth took place.	Cesarean delivery; Vaginal delivery.
Birth weight	Weight in grams taken up to the 5th hour after birth.	<2,500 grams (low weight); ≥2,500 grams (normal weight).
Gestational age	Number of weeks of gestation at the time of birth.	Preterm (<37 weeks); Term (≥37 weeks).
Type of pregnancy	Number of conceptuses per pregnancy.	Single; Twins or more.
Socioeconomic and demographic variables		
Maternal education	Degree of maternal education in years of study completed.	<1 year; 1 to 3 years; 4 to 7 years; 8 or more years.
Maternal civil status		Married; Divorced; Single; Consensual union; Widow.

CID-10: Tenth Revision of the International Statistical Classification of Diseases and Related Health Problems.

This study waived the need for consideration by the Ethics Committee for Research on Human Beings (CEP), considering that the data used are from an open access Brazilian information system, available on the website of the IT Department of the Unified Health System (DATASUS) (http://www.datasus.gov.br), in which the data are presented without identifying the subjects.

RESULTS

Between 1999 and 2020, there were 65,277,959 live births registered in SINASC. Of these, 33,699 were born with OC, occurring in a proportion of 17.8% (6,022) cases of OC associated with other congenital malformations, and 82.1% (27,677) with isolated OC. Regarding the cases of isolated OC, the majority were CLP (9,619 or 34.7%), followed by CP (9,442 or 34.1%), and by CL (8,616 or 31.1%). The categorical descriptive analysis of these NSOC cases, according to biological, maternal and child health care and socioeconomic/demographic characteristics, can be seen in Tables 2 and 3. In a quantitative analysis, the maternal and paternal mean age were 26 (±6.99) and 31 (±7.79) years, respectively, and the children's mean weight at birth was 2,933 kg (±715.5). The bivariate analysis showed a statistic significant crude association between the sex and the NSOC types occurrence (Table 4).

At the national level, the prevalence of OC found was 5.16/10,000, and 4.24/10,000 regarding the non-syndromic cases (Figure 1). Also, the prevalence of isolated OC was analyzed at state and federal levels. For the regions, the prevalence was calculated for each year and for the overall period. The South and Southeast regions of Brazil had the highest prevalence — respectively, 6.34/10,000 and 4.26/10,000 —, while the lowest were recorded in the Northeast, with 3.52/10,000, and the North region, with 3.66/10,000 live births. In the Midwest, the prevalence of non-syndromic OC was 4.05/10,000. The state prevalence temporal trend of isolated OC for each year can be found in Figure 2. The prevalence for the period was also calculated for the Federative Units (Table 5).

DISCUSSION

The results of this study showed that the epidemiological profile of live births with OC in Brazil: individuals with CLP, males, with white race/color, who were born in hospitals with birthweight ≥2500 g and gestational age ≥37 weeks, by cesarean section, with Apgar scores ≥7. Cleft cases were more frequent among mothers who were in their first pregnancy, with single pregnancies, and had seven or more prenatal appointments. These mothers were between 20 and 29 years old, had eight to

Table 2. Number of live births with orofacial cleft according to biological and socioeconomic characteristics in Brazil between 1999 and 2020

	Total (n)	Total (%)
Type of orofacial cleft		
Cleft lip	8616	31.1
Cleft lip with palate	9619	34.7
Total	27,677	100.0
Sex of newborn		
Female	11,380	41.1
Male	16,276	58.8
Total	27,656	100.0
Ethnicity		
White	13,847	52.4
Black	907	3.4
Asian	102	0.3
Brown	11,329	42.9
Indigenous	206	0.7
Total	26,391	100.0
Maternal age (years old)		
≤19	4975	17.9
20–29	13,469	48.6
30–34	4975	17.9
35–39	3106	11.2
40 years or more	1137	4.1
Total	27,662	100.0
Paternal age (years old)		
≤19	234	4.4
20–39	4301	81.9
40 years or more	715	13.6
Total	5250	100.0
1st minute Apgar score		
≥7	21,632	96.7
<7	729	3.2
Total	22,361	100.0
5th minute Apgar score		
≥7	24,797	97.0
<7	742	2.9
Total	25,539	100.0
Maternal civil status		
Single	12,673	4.3
Married	9910	36.2
Consensual union	4332	15.8
Divorced	333	1.2
Widow	72	0.2
Total	27,320	100.0
Maternal educational level (years)		
None	479	1.76
1 to 3	2063	7.57
4 to 7	7656	28.1
8 to 11	12,713	46.6
12 years or more	4336	15.9
Total	27,247	100.0

Table 3. Number of live births with orofacial cleft according to maternal and child health care characteristics in Brazil between 1999 and 2020.

	Total (n)	Total (%)
Number of previous pregnancies		
None	9301	37.6
One	5450	22.0
Two or more	9943	40.2
Total	24,694	100.0
Number of prenatal appointments		
None	681	2.4
1 to 3	2275	8.3
4 to 6	7814	28.6
7 or more	16,542	60.5
Total	27,312	100.0
Place of delivery		
Hospital	27,203	98.3
Home	183	0.6
Others	55	0.2
Other health place	230	0.8
Total	27,671	100.0
Type of delivery		
Vaginal	12,812	46.4
Cesarean	14,814	53.6
Total	27,626	100.0
Birth weight		
≥2500 g	21,570	77.9
<2500 g	6102	22.0
Total	27,672	100.0
Gestational age		
≥37 weeks	23,425	85.7
<37 weeks	3892	14.2
Total	27,317	100.0
Type of pregnancy		
Single	26,988	97.7
Twin or more	644	2.3
Total	27,632	100.0

eleven years of study and were single. The prevalence of NSOC in Brazil was 4.24/10,000. For the Brazilian regions, the South and Southeast had the highest mean prevalence of OC, while the lowest were recorded in the Northeast and North. In relation to the Federative Units, the highest and lowest prevalences of clefts were found, respectively, in Paraná and Acre.

Regarding biological variables, the findings of this study agree with previous ones that also found a higher occurrence of CLP than CP in Brazilian.[18-20] Also, non-syndromic OC was more frequent in males,[2,21-24] with a predominance of satisfactory 1st and 5th Apgar scores.[22] The bivariate analysis found a statistically significant association between sex and OC type, showing a higher occurrence of CP among women and CL/P among men, as in previous studies.[19,20] For maternal age, there is no consensus in the literature. Although the age group from 20 to 29 years was more predominant in this and other studies,[14,15,23] the literature also points to a higher occurrence of OC in children whose mothers were of intermediate age, up to 34 years of age,[16,22,23] and advanced age, from 35 years onwards.[25] Maternal age differences may have occurred due to possible methodological differences in the studies or possible confounding factors in the analyzed populations. The same occurs for the assessment of ethnicity. Most of those born with any type of non-syndromic OC in Brazil were self-reported as white, in line with previous studies.[24,25] However, others[15,22,24] found a higher occurrence of OC in non-white children, who, due to great social inequality, would be distributed in low-income social strata, thus having less access to the health system. These findings may be related to the effect of racial miscegenation in Brazil in the genotype determination, and the lack of standardization in the field of ethnic classification in the Live Birth Declaration.[24,25] Thus, it is recommended that future studies be carried out to better understand the role of parents' age and ethnicity in the occurrence of OC.

The epidemiological profile of OC based on maternal and child health care variables was similar to that found by other descriptive studies.[22,24,25] Among the live births with OC, most of the mothers were in the first, single pregnancy and had had seven or more prenatal consultations. Authors[22] mention that women, upon receiving the diagnosis of OC in the fetus, decide

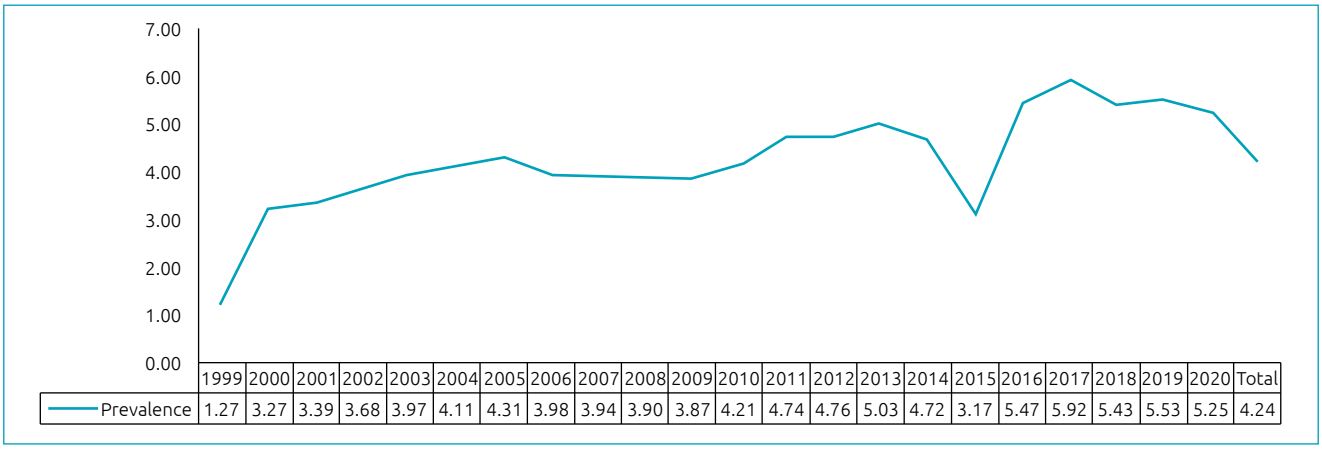
Table 4. Crude association between sex and non-syndromic orofacial cleft types in Brazil between 1999 and 2020.

	Cleft palate		Cleft lip		Cleft lip with palate		p-value
	(n)	(%)	(n)	(%)	(n)	(%)	
Female	4446	39.0	3267	28.7	3667	32.2	<0.001
Male	4989	30.6	5341	32.8	5946	36.5	
Total	9435	34.1	8608	31.1	9613	34.7	

to carry out more prenatal consultations, or that, when they perform more prenatal appointments, they identify the presence of the malformation and decide to interrupt the pregnancy. It was also found that most children with OC were born by cesarean section, at term and with a weight considered normal. However, this finding may also be associated with the fact that Brazil has a high number of cesarean deliveries, being the second in the top five countries with the highest cesarean section rate worldwide (55.7%).[26] The fact that the majority of the children born were term reveals normal weight and length gain. In addition, a higher occurrence of normal weight[23,24] in those born with OC favors their adequacy to extrauterine life, since mortality rates in this weight range are lower.[22]

With regard to socioeconomic and demographic variables, the profile of OC was in agreement with the literature.[14,16,22,23,27] Despite the distribution of mothers with an average education level among those born with OC in Brazil, it is known that low schooling is a predominant Brazilian characteristic in the profile of neonatal deaths.[28]

Only five[6-8,28,29] studies were found in the literature regarding the prevalence of OC using SINASC data for the entire country, but none of them covered the entire time period selected for the present analysis. For the period between 1998 and 2002, Rodrigues et al.[6] found a prevalence of 3.6/10,000 live births. Studies done involving the years between 2009 and 2013 found prevalences ranging from 4.85/10,000 live births[29] to 5.86/10,000 live births.[28] The most recent ones[7,8] found prevalences near 5.1/10,000 live births for the 2005–2017 period. In all of these studies the prevalence of OC showed differences between the Federative Units and the regions. The overall prevalence of OC in Brazil found in this present study is in accordance with what was found in the literature regarding the country;[6-8,28,29] however,



	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	Total
Prevalence	1.27	3.27	3.39	3.68	3.97	4.11	4.31	3.98	3.94	3.90	3.87	4.21	4.74	4.76	5.03	4.72	3.17	5.47	5.92	5.43	5.53	5.25	4.24

Figure 1. Prevalence of non-syndromic orofacial cleft in Brazil (per 10,000 livebirths), by year.

	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
North	0.56	2.61	2.44	2.92	3.34	3.17	3.68	3.87	2.92	3.14	3.93	4.37	4.65	3.70	3.86	3.70	4.08	4.98	4.96	5.07	4.27	3.91
Northeast	1.00	2.13	2.10	2.60	2.46	2.68	2.68	2.78	3.07	3.08	3.34	3.71	4.42	4.28	4.63	4.55	4.21	4.94	5.16	5.03	5.10	4.87
Midwest	0.81	2.36	3.82	3.35	3.98	4.05	3.24	3.43	3.30	3.28	2.59	4.67	5.74	4.82	5.03	4.90	5.05	4.39	4.55	4.92	5.31	5.16
Southeast	1.53	3.38	3.59	4.08	4.46	4.94	5.40	4.26	4.10	4.03	3.62	3.34	3.79	4.43	4.83	4.44	2.08	5.56	6.35	5.17	5.61	5.45
South	1.68	6.23	6.18	5.69	6.60	5.70	6.02	6.38	6.83	6.49	6.63	7.60	7.83	7.62	7.42	6.66	2.39	7.35	7.87	7.60	7.38	6.61

Figure 2. Prevalence of non-syndromic orofacial cleft in Brazil and Regions (per 10,000 livebirths), by year.

the Brazilian prevalence of this malformation is lower than the global, which is about 1–1.5/1,000 live births.[3] Some reasons that could explain it are the underestimation of reported cases of OC in the national system, and the misdiagnosis of clefs in some Brazilian regions, especially those with the lowest reports.[30] Also, SINASC only collects information about live births, so if a child dies during birth or even prior to delivery and she/he has OC this case will not be registered.[29]

Regarding the annual prevalence of OC in Brazil, there has been an increase over the years, with fluctuations in the Brazilian regions, as also found by other authors.[7] The year 1999 recorded the lowest prevalence rate in the country and in the regions, possibly due to the fact that this was the year in which information on malformations began to be collected and which possibly had the highest occurrence of underreporting, which can be evidenced by the absence of records of OC occurrence in some Brazilian states (Acre, Amapá, Mato Grosso, Mato Grosso do Sul, Pará, Rio Grande do Norte and Roraima). In 2015, there was a decline in the annual prevalence rate of OC in the country, possibly due to a lack of OC records that year in Santa Catarina and São Paulo, which are states with significant numbers of cases recorded over the years. When evaluating the annual behavior of clefts by region, this one-off drop in prevalence in 2015 was also more expressive in the South and Southeast regions, which the aforementioned states are part of.

Table 5. Prevalence of non-syndromic orofacial cleft for each Federative Unit from 1999 to 2020 (per 10,000 livebirths).

Federative Unit	Prevalence
Rondônia	5.07
Acre	1.86
Amazonas	3.39
Roraima	4.60
Pará	3.83
Amapá	1.89
Tocantins	3.83
Maranhão	2.13
Piauí	2.38
Ceará	3.95
Rio Grande do Norte	4.97
Paraíba	3.81
Pernambuco	5.47
Alagoas	2.21
Sergipe	4.50
Bahia	2.76
Minas Gerais	3.56
Espírito Santo	3.72
Rio de Janeiro	4.83
São Paulo	4.40
Paraná	6.44
Santa Catarina	6.04
Rio Grande do Sul	6.41
Mato Grosso do Sul	2.87
Mato Grosso	4.90
Goiás	4.57
Distrito Federal	3.08

Analyzing the regions most and least affected by clefts, the results were also consistent with other studies.[6,7,28,29] This difference between regions can be explained by the greater probability of underreporting of cases in the economically poorer regions,[8] since, when the OC were evaluated by state, an absence of OC records was found for some years, especially in the Northern states.

Regarding the Federative Units, the prevalence of OC showed disparities, with the highest and lowest found, respectively, in Paraná and Acre. The absence of clefts records in 16 states over the period analyzed may have contributed to these disparities. In addition to the years 1999 and 2015, that do not have records of OC cases in some Federative Units, in 2000 and 2007 there were no OC cases registered in Amapá, in 2001 there were no OC cases in Roraima, and in 2003 there were no records of clefts cases in Acre and Amapá.

Some limitations, typical of the descriptive study model, do not allow this study to establish associations of causal inferences between the analyzed variables and the etiology of these malformations; they only make possible to direct actions (of care, prevention and control) and to formulate causal hypotheses to be tested by analytical studies. The period of time covered in this study was limited to 2020 due to the data availability in the system, which only publishes data regarding the two years previous to the current year. In addition, the main limitations of the present study are related to underreporting and/or errors in filling in the Live Birth Declaration and consequently in SINASC, possibly caused by the turnover and lack of preparation of health professionals to deal with these data. Therefore, maximum completeness should be sought when filling in, and this requires a joint effort from all professionals involved in this process to further increase the credibility of this information.

In Brazil, descriptive studies on OC are usually specific, describing this event in a specific city, state, region or health service. There are few studies with population data in a national context, such as the one reported in the present study. Thus, the results presented here make it possible to direct some prevention

and care actions directed at OC and point out the need for analytical studies to assess a possible association between environmental factors and the emergence of these malformations.

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Conflict of interests

The authors declare there is no conflict of interests.

Authors' contributions

Study design: Silva AM, Freitas VS. *Data collection*: Silva AM, Calumby RT, Freitas VS. *Data analysis*: Silva AM, Calumby RT, Freitas VS. *Manuscript writing*: Silva AM, Calumby RT, Freitas VS. *Manuscript revision*: Silva AM, Freitas VS. *Study supervision*: Freitas VS.

Declaration

The database that originated the article is available with the corresponding author.

REFERENCES

1. Nagappan N, John J. Sociodemographic profile of orofacial cleft patients in India: a hospital-based study. Int J Med Public Health. 2015;5:35-9. https://dx.doi.org/10.4103/2230-8598.151253
2. Sandy J, Davies A, Humphries K, Ireland T, Wren Y. Cleft lip and palate: care configuration, national registration, and research strategies. J World Fed Orthod. 2020;9:S40-4. https://doi.org/10.1016/j.ejwf.2020.09.003
3. Mossey PA, Shaw WC, Munger RG, Murray JC, Murthy J, Little J. Global oral health inequalities: challenges in the prevention and management of orofacial clefts and potential solutions. Adv Dent Res. 2011;23:247-58. https://doi.org/10.1177/0022034511402083
4. IPDTOC Working Group. Prevalence at birth of cleft lip with or without cleft palate: data from the International Perinatal Database of Typical Oral Clefts (IPDTOC). Cleft Palate-Craniofacial J. 2011;48:66-81. https://doi.org/10.1597/09-217
5. Kadir A, Mossey PA, Blencowe H, Moorthie S, Lawn JE, Mastroiacovo P, et al. Systematic review and meta-analysis of the birth prevalence of orofacial clefts in low- and middle-income countries. Cleft Palate Craniofac J. 2017;54:571-81. https://doi.org/10.1597/15-221
6. Rodrigues K, Sena MF, Roncalli AG, Ferreira MA. Prevalence of orofacial clefts and social factors in Brazil. Braz Oral Res. 2009;23:38-42. https://doi.org/10.1590/s1806-83242009000100007
7. Shibukawa BM, Rissii GP, Higarashi IH, Oliveira RR. Fatores associados à presença de fissura labial e/ou fenda palatina em recém-nascidos brasileiros. Rev Bras Saude Mater Infant. 2019;19:957-66. https://doi.org/10.1590/1806-93042019000400012
8. Silva RS, Macari S, Santos TR, Werneck MA, Pinto RS. The panorama of cleft lip and palate live birth in Brazil: follow-up of a 10-year period and inequalities in the health system. Cleft Palate Craniofac J. 2022;59:1490-501. https://doi.org/10.1177/10556656211050004
9. Denadai R, Lo L. The burden of orofacial clefts: global trends and focus on underresourced regions. In: Denadai R, Lo L, eds. Current concept in cleft surgery. Singapore: Springer; 2022. p. 13-20.
10. Agbenorku P. Orofacial clefts: a worldwide review of the problem. Int Sch Res Notices. 2013;2013:1-7. https://doi.org/10.5402/2013/348465
11. Figueiredo JC, Ly S, Magee KS, Ihenacho U, Baurley JW, Sanchez-Lara PA, et al. Parental risk factors for oral clefts among Central Africans, Southeast Asians, and Central Americans. Birth Defects Res A Clin Mol Teratol. 2015;103:863-79. https://doi.org/10.1002/bdra.23417
12. Chiavarini M, Lanari D, Minelli L, Salmasi L. Socio-demographic determinants and access to prenatal care in Italy. BMC Health Serv Res. 2014;14:174. https://doi.org/10.1186/1472-6963-14-174
13. Kruppa K, Krüger E, Vorster C, van der Linde J. Cleft lip and/or palate and associated risks in lower-middle-income countries: a systematic review. Cleft Palate Craniofac J. 2022;59:568-76. https://doi.org/10.1177/10556656211018952
14. Moura JR. Perfil clínico-epidemiológico dos portadores de fissuras orofaciais em residentes do estado da Bahia: um estudo descritivo [dissertation]. Feira de Santa: Universidade Estadual de Feira de Santana; 2014.
15. Viena CS, Sá J, Veiga PC, Coletta R, Medrado AP, Reis SR. Epidemiological study of cleft palate in the state of Bahia, Brazil. Brazilian J Med Hum Heal. 2017;5:123-33. https://doi.org/10.17267/2317-3386bjmhh.v5i4.1337
16. Alves LD, Moura JR, Freitas VS. Análise epidemiológica das fissuras labiais e/ou palatinas no município de Feira de Santana: estudo de corte transversal. J Dent Pub H. 2019;10:24-32. https://doi.org/10.17267/2596-3368dentistry.v10i1.2244
17. Bastos Neto BC. Prevalência de nascidos vivos com fissura labiopalatina no estado da Bahia, 1994 a 2015 [monography]. Governador Mangabeira: Faculdade Maria Milza; 2019.
18. Maranhão SC, Sá J, Cangussú MC, Coletta RD, Reis SR, Medrado AR. Nonsyndromic oral clefts and associated risk factors in the state of Bahia, Brazil. Eur Arch Paediatr Dent. 2021;22:121-7. https://doi.org/10.1007/s40368-020-00522-0

19. Errari-Piloni C, Barros LA, Jesuíno FA, Valladares-Neto J. Prevalence of cleft lip and palate and associated factors in Brazil's midwest: a single-center study. Braz Oral Res. 2021;35:e039. https://doi.org/10.1590/1807-3107bor-2021.vol35.0039

20. Monlleó IL, Barros AG, Fontes MI, Andrade AK, Brito GM, Nascimento DL, et al. Diagnostic implications of associated defects in patients with typical orofacial clefts. J Pediatr (Rio J). 2015;91:485-92. https://doi.org/10.1016/j.jped.2014.12.001

21. Matos FG, Santos KJ, Baltazar MM, Fernandes CA, Marques AF, Luz MS. Perfil epidemiológico das fissuras labiopalatais de crianças atendidas em um centro de referência paranaense. Rev Enferm UFSM. 2020;10:1-14. https://doi.org/10.5902/2179769238654

22. Rodrigues LS, Lima RH, Costa LC, Batista RF. Características das crianças nascidas com malformações congênitas no município de São Luís, Maranhão, 2002-2011. Epidemiol Serv Saude. 2014;23:295-304. https://doi.org/10.5123/S1679-49742014000200011

23. Andrade NM, Fernandes TV, Catão ES, Silva GC, Fernandes LH, Cavalcanti AF, et al. Prevalence of cleft lip and palate in Brazilian children 2011–2015. Internet J Med Updat. 2018;13:10-4. https://doi.org/10.4314/ijmu.v13i1.3

24. Cavalcanti AL, Catão ES, Fernandes TV, Andrade NM, Silva GC, Fernandes LH, et al. An ecological study on orofacial clefts in northeastern Brazil. Acta Facultatis Medicae Naissensis. 2019;36:102-9. https://doi.org/10.5937/afmnai1902102L

25. Figueirêdo CJ, Vasconcelos WK, Maciel SS, Maciel WV, Gondim LA, Tassitano RM. Prevalence of oral clefts in the state of Rio Grande do Norte, Brazil, between 2000-2005. Rev Paul Pediatr. 2011;29:29-34. https://doi.org/10.1590/S0103-05822011000100005

26. Betran AP, Ye J, Moller AB, Souza JP, Zhang J. Trends and projections of caesarean section rates: global and regional estimates. BMJ Glob Health. 2021;6:e005671. https://doi.org/10.1136/bmjgh-2021-005671

27. Ponton E, Courtemanche R, Singh TK, Duffy D, Courtemanche DJ, Loock C. Assessing the social determinants of health and adverse childhood experiences in patients attending a children's hospital cleft palate-craniofacial program. Cleft Palate Craniofac J. 2022;59:1482-9. https://doi.org/10.1177/10556656211048742

28. Sousa GF, Roncalli AG. Orofacial clefts in Brazil and surgical rehabilitation under the Brazilian National Health System. Braz Oral Res. 2017;31:e23. https://doi.org/10.1590/1807-3107BOR-2017.vol31.0023

29. Abreu MH, Lee KH, Luquetti DV, Starr JR. Temporal trend in the reported birth prevalence of cleft lip and/or cleft palate in Brazil, 2000 to 2013. Birth Defects Res A Clin Mol Teratol. 2016;106:789-92. https://doi.org/10.1002/bdra.23528

30. Freitas e Silva DS, Mauro LD, Oliveira LB, Ardenghi TM, Bönecker M. Estudo descritivo de fissuras lábio-palatinas relacionadas a fatores individuais, sistêmicos e sociais. RGO (Porto Alegre). 2008;56:387-91.

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Coast-or Inland Residence and Differences in the Occurrence of Cleft Lip and Cleft Palate

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Abstract

Objetive: To analyze if differences in lifestyle and environment between coastal and inland areas are associated with differences in frequency of orofacial cleft types.

Design: Populational cross-sectional study.

Setting: All live borns with orofacial cleft registered at Brazilian Live Birth Information System between 1999 and 2020.

Participants: 33,699 live borns with orofacial cleft.

Intervention: Data from borns with orofacial cleft were collected at Brazilian Live Birth Information System.

Main outcome measure: Differences in frequencies between the cleft types and covariates were determined using chi-square. Bivariate analysis was done to obtain the prevalence ratio of types of clefts by geographic origin. Multiple logistic regression analysis was used to determine adjusted odds ratios, controlling for covariates, establishing a significance level of p value <0.05.

Results: The frequency of cleft types was statistically significant different according to geographic origin (inland x coast). For syndromic clefts, the prevalence ratio for cleft lip with/without palate was 3.6 times higher inland (p value = 0.000). Regarding non-syndromics, the prevalence ratio for cleft lip with/without palate was two times higher inland (p value = 0.000). Logistic regression suggested cleft lip with/without palate was 6.33 more likely to occur in inland regions (p value = 0.000).

Conclusion: Geographic origin was associated with the type of cleft in Brazil, with a higher prevalence of cleft lip with/without palate in inland areas, compared to cleft palate, which was higher in the coast.

Keywords

cleft lip and palate, cleft lip, epidemiology

Introduction

Orofacial clefts (OC) are common congenital malformations caused by genetic and environmental factors that can act together or in isolation during the first trimester of intrauterine development, resulting generally in deformities of the lip and/or palate.[1,2] They constitute a serious burden worldwide due to the aesthetic, functional, and emotional alterations caused in the affected individuals.[3]

The most well-known classification for clefts divides them into cleft lip with or without palate (CL + P) and cleft palate only (CP) due to their embryological and epidemiological distinctions.[4] Additionally, OC can be classified based on the presence or absence of other malformations or anomalies, respectively, as syndromic (SOC) or non-syndromic/isolated (NSOC) clefts.[5]

SOC represents a genetic defect (monogenic or chromosomal aberration), and the majority of their causal genes have been successfully identified.[6] On the other hand, 70% of all clefts are NSOC,[7] which have a multifactorial etiology resulting from both genetic and environmental factors identified.[6] Socioeconomic status, family, neighborhood, and social network context are some environmental factors that

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can influence epigenetic regulation through DNA methylation, histone modifications, and non-coding RNAs, thereby altering gene expression and resulting in clefts.[8,9]

Our hypothesis is that differences in lifestyle and the environment that exist between geographic living areas (coast vs. inland, rural vs. urban) are associated with differences in the frequency of OC.[10–12] These differences are generally related to geographic characteristics, such as air and water quality, health services and food availability, green space, and working conditions.[8] Based on that, the objective of this study was to analyze whether differences in lifestyle and being born in coastal or inland areas are associated with differences in the frequency of OC types.

Materials and Methods

This is a population-based cross-sectional study. The study included 33,699 live births with OC registered in the Brazilian Live Birth Information System (SINASC) between 1999 and 2020. The STROBE guidelines for reporting observational studies were used. The investigators involved had no contact with human subjects, and the data used are from an open-access Brazilian information system available online,[13] in which the data are disseminated without identifying the subjects.

Data on cleft type, geographic origin of the patient (coast or inland), presence of associated congenital anomalies or syndromes, sex of the newborn, ethnicity, Apgar score in the 1st minute, Apgar score in the 5th minute, maternal age, paternal age, maternal parity, birth weight, type of pregnancy, and the number of prenatal appointments were recorded. All variables were previously analyzed according to the incompleteness criteria proposed by Romero and Cunha 13,14: excellent (< 5%), good (5% to 9.9%), regular (10% to 19.9%), poor (20% to 49.9%), and very poor (≥ 50%). Those with a poor or very poor degree of completeness were removed from the study. Following this classification, the variable 'geographic origin of the patient' had 100% completeness, while the others ranged from 89.22% (parity) to 99.94% (maternal age). The only exception was information about paternal age, which was missing in 81% of the records; due to its very poor degree of completeness, this variable was not included in the study.

The OC types adopted by SINASC follow the Tenth Revision of the International Statistical Classification of Diseases and Related Health Problems (CID-10),[14] which groups cleft types and subtypes into Q35 (cleft palate - CP), Q36 (cleft lip – CL), and Q37 (cleft lip and palate - CLP). Live births that were coded for both CL and CP simultaneously in SINASC were considered as having CLP. Later, cases were divided into CP and cleft lip with or without palate (CL + P). The geographic origin was divided into two main categories: coast and inland, based on the IBGE classification.[15]

The data were submitted to statistical analysis using STATA (Software for Statistics and Data Science) version 10 software, establishing a significance level of $p < 0.05$. CP was used as the reference dependent variable, and the inland region was considered as the exposure. Continuous variables were later categorized. Covariates included in the analysis were the sex of the infant, maternal age, and parity, as determined based on the literature.[11] Differences in frequencies between the cleft types and the covariates were tested using the chi-square test. Bivariate analysis was developed to obtain the crude prevalence ratio (PR) for the relationship between geographic origin and type of cleft. Multiple logistic regression analysis was performed to determine the adjusted odds ratios (ORs) for the same relationship while controlling for covariates.

Data were submitted to statistical analysis using the STATA (Software for Statistics and Data Science) version 10 software, establishing a significance level of $p < 0.05$. CP was used as dependent variable of reference, and inland region as exposition. Continuous variables were later categorized. Covariates included in the analysis were sex of the infant, maternal age, parity, determined based on the literature.[11] Differences in frequencies between the cleft types and the covariates were tested using the chi-square test. Bivariate analysis was developed in order to obtain the crude prevalence ratio (PR) for the relation between geographic origin and type of cleft. Multiple logistic regression analysis was performed to determine the adjusted ODDs for this same relation while controlling for covariates.

Results

Based on the data collected from SINASC, there were 33,699 cases of OC registered in Brazil between 1999 and 2020; 6022 (17.9%) were syndromic (SOC), and 27,677 (82.1%) were isolated (NSOC). The analysis for both types revealed that the mean maternal age was 26 (±7) years, the mean Apgar scores at 1st and 5th minutes were 7.5 (±2) and 8.8 (±2), respectively, and the mean birth weight was 2933 (±715) grams.

The frequency of cleft types was statistically significantly different according to geographic origin (inland x coast) for both syndromic and isolated cases (Figure 1). Among SOC cases, CP was more frequent at the coastline (86%), and CL + P was more equally distributed, occurring in 47% of cases inland. The bivariate analysis for these cases showed that the crude prevalence ratio for CL/P was 3.6 times higher among those born with clefts inland (p value = 0.000, OR = 3.6, 95% confidence interval 2.37 - 5.61). Regarding NSOC, CP was more common at the coastline (69%), and CL + P was more common inland (68%). NSOC showed a crude prevalence ratio for CL + P that was two times higher among those born with clefts inland (p value = 0.000, OR = 2.0, 95% confidence interval 1.99–2.34).

NSOC types showed statistically significant differences in their geographic distribution (coast vs. inland) by sex, maternal age, and parity. In terms of sex, they occurred in higher proportions in males in both inland and coastal regions (65% and 62%, respectively), while CP was more equally distributed in both regions, representing approximately 47% of the cases in

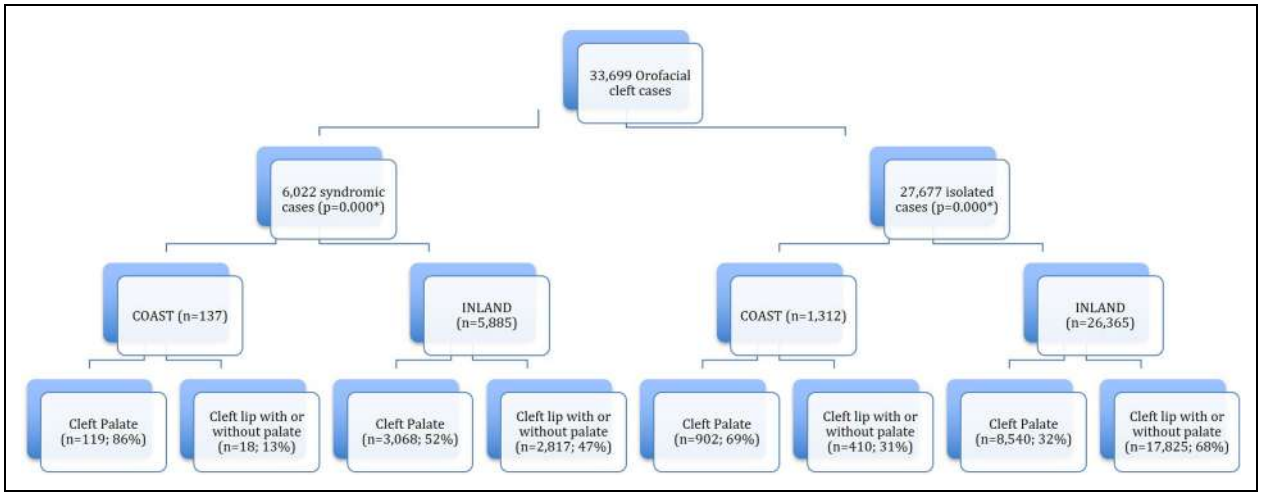

Figure 1. Frequency of orofacial clefts according to type and geographic origin. **Indicates statistically significant difference.*

each area. Regarding maternal age, in both groups of clefts, the majority of the mothers were younger than 35 years (84.66%). In inland regions, the frequencies of mothers younger than 35 years were 84% (CP) and 95% (CL + P), respectively. At the coast, these frequencies were similar, 82% (CP) and 85% (CL + P), respectively. Most mothers were in their first or second pregnancy, but there was a difference in parity between cleft types according to the geographic region. While children with CL + P were more frequent in coastal areas among mothers in their first or second pregnancy (98% at the coast and 72% inland), children with CP were more frequent inland among those in their third or subsequent pregnancies (68% at the coast and 78% inland).

For NSOC, there were statistically significant differences in the distribution of sex, maternal age, and parity between the two types of clefts tested (Table 1). Due to this, they were included in the logistic regression to adjust for their influence as covariates. After applying backward selection, only maternal parity was retained in the regression model. This logistic regression model showed that CL + P was six times more likely in inland regions (p-value = 0.000; OR = 6.33, 95% confidence interval 5.43-7.37). The receiver operating characteristic (ROC) curve for the prediction model was 0.764, indicating good discrimination of the model between individuals with CP and CL + P (Figure 2).

Discussion

In the present study, geographic origin was associated with the type of OC in Brazil, with a higher prevalence ratio of CL + P occurrence in inland areas compared to CP, which had a higher prevalence ratio in coastal areas. Also, among the covariates studied here, only parity could partly explain the differences in frequency between cleft types along the coast and inland.

Few studies have focused on studying the relationship between geographic origin and OC.[16] In Brazil, only one other study, which used data from a single state, attempted to investigate this relationship and also found that the frequency of CL + P was higher in inland areas, while CP prevalence was higher in coastal areas.[11] A few other studies have focused on classifying geographic origin based on rural vs. urban areas. A case-control study conducted to explore the association between maternal residence's region (rural x urban) and the risk of a child developing OC in Washington State, US, found that babies born to women residing in rural regions had a greater likelihood of developing NSOC compared to those born to women living in urban areas.[12] Similarly, an ecological study aimed at investigating the association of CL + P and CP with urban and rural residence in Texas, US, discovered that living in more rural areas was linked to an increased adjusted risk of CL + P.[10]

These results further strengthened our hypothesis that differences in lifestyle and environment, such as maternal smoking, antiepileptic drug use, stress, and nutritional deficiencies, which exist between geographic living areas (coast vs. inland; rural vs. urban), are associated with differences in the frequency of OC. This association is especially relevant in less resourced countries, where these factors tend to be more frequent, increasing the likelihood of them modifying gene function in various cleft types.

In this study, it was more likely for the child to have higher parity (third or more pregnancies) if he or she was born with CP in inland regions. This finding agrees with a previous one that found higher parity for children who were born inland.[11] In the literature, parity has been associated with OC,[17] but it appeared to explain very little of the difference in frequencies between inland and coastal areas.[11]

Some limitations inherent to cross-sectional studies are present in this study, such as the challenge of determining causality when exposure and outcome are measured simultaneously. However, this type of study is designed to test associations, which aligns with the purpose of this investigation.[18] Other limitations to note include variability in the

Table 1. Crude Associations Between the Covariates and Cleft Types.

Variable	Cleft Palate		Cleft Lip with/without Palate		Total		p value
	(n)	(%)	(n)	(%)	(n)	(%)	
Sex							0.000*
Female	4446	47.12	6934	38.05	11,380	41.15	
Male	4989	52.88	11,287	61.95	16,276	58.85	
Total	9.435	100.00	18.221	100.00	27.656	100.00	
Maternal age							0.000*
< 35 years	7792	82.64	15,627	85.71	23,419	84.66	
≥ 35 years	1637	17.36	2606	14.29	4243	15.34	
Total	9429	100.00	18,233	100.00	27,662	100.00	
Ethnicity							0.102
Non white	4347	48.23	8197	47.17	12,544	47.53	
White	4666	51.77	9181	52.83	13,847	52.47	
Total	9013	100.00	17,378	100.00	26,391	100.00	
Type of pregnancy							0.633
Single	9212	97.73	17,776	97.64	26,988	97.67	
Twin or more	214	2.27	430	2.36	644	2.33	
Total	9426	100.00	18,206	100.00	27,632	100.00	
Number of prenatal appointments							0.042
≥7 appointments	5564	59.73	10,978	61.00	16,542	60.57	
<7 appointments	3751	40.27	7019	39.00	10,770	39.43	
Total	9315	100.00	17,997	100.00	27,312	100.00	
Parity							0.000*
First or second pregnancy	1473	22.78	13,278	72.84	14,751	59.73	
Third or more	4993	77.22	4950	27.16	9943	40.27	
Total	6466	100.00	18,228	100.00	24,694	100.00	

*Indicates statistically significant difference.

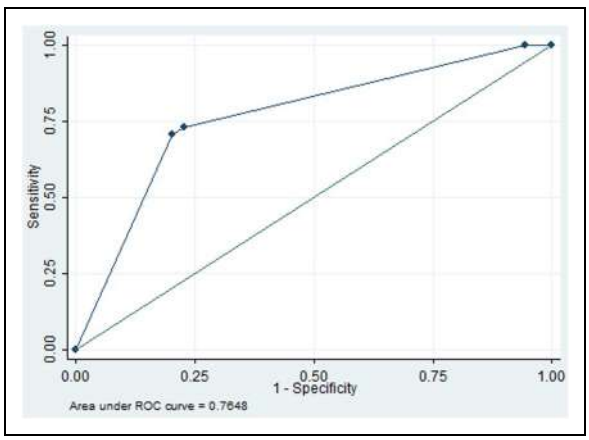

Figure 2. Receiver operating characteristic (ROC) curve from the logistic regression model for cleft type, geographic origin, sex, maternal age and parity.

degree of incompleteness in the SINASC variables, which could introduce regional differences in data quality and potentially affect the analyses conducted with this data.[19] Nevertheless, the variables included in this study generally exhibited an excellent degree of incompleteness (< 5%), and the results can be considered acceptable for the country. The exception was parity, which fell within the regular range of incompleteness (10.78%). It was retained in the analysis due to its relevance in previous studies.[11] Yet, rare cleft could not be studied separately here because this type of cleft is presented on the Spina Classification.[20] CID-10 includes rare cases, but classifies clefts types and its subtypes in a codification system that differ from the Spina Classification. Additionally, the years of the study were limited to 2020 as that was the last available year of data in the system at the time of consultation. However, this limitation does not diminish the significance of our findings since our analysis covers a span of 21 years, and existing evidence supports the representativeness of SINASC for the Brazilian population.[19]

This study aimed to test the association between geographic origins (coast vs. inland) and differences in the frequency of OC. Inequalities, such as those related to lifestyle and environmental background between geographic regions, can act as social and environmental stressors that modify the way in which genes are expressed and ultimately alter our risk for disease.[21] CP was more common at the coastline, while CL + P was more common inland. Due to the complex etiology of OC, investigations into how environmental factors are related to OC occurrence could help researchers understand how those factors influence gene expression through epigenetic changes.[22]

Conclusion

The geographic origin was associated with the type of orofacial cleft in Brazil, with a higher prevalence ratio of CL + P occurrence in inland areas compared to CP prevalence ratio, which was higher on the coast. Also, among the covariates studied here, only parity could partly explain the differences in frequency between cleft types on the coast vs. inland.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Disclosure Statement

Investigators here had no contact with human subjects and the data used are from an open access Brazilian information system, available online (http://www.datasus.gov.br), in which the data are disbursed without identifying the subjects.

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References

1. Mossey PA, Shaw WC, Munger RG, Murray JC, Murthy J, Little J. Global oral health inequalities: Challenges in the prevention and management of orofacial clefts and potential solutions. *Adv Dent Res*. 2011;23(2):247-258. doi:10.1177/0022034511402083
2. Marazita ML. The evolution of human genetic studies of cleft lip and cleft palate. *Annu Rev Genomics Hum Genet*. 2012;13:263-283. doi:10.1146/annurev-genom-090711-163729
3. Denadai R, Lo LJ. The burden of orofacial clefts: global trends and focus on underresourced regions. In: *Current concept in cleft surgery*. Springer; 2022:13-20. doi:doi.org/10.1007/978-981-19-3163-5_2
4. Luijsterburg AJM, Rozendaal AM, Vermeij-Keers C. Classifying common oral clefts: A new approach after descriptive registration. *Cleft Palate-Craniofacial Journal*. 2014;51(4):381-391. doi:10.1597/12-088
5. Watkins SE, Meyer RE, Strauss RP, Aylsworth AS. Classification, epidemiology, and genetics of orofacial clefts. *Clin Plast Surg*. 2014;41(2):149-163. doi:10.1016/j.cps.2013.12.003.
6. Alade A, Awotoye W, Butali A. Genetic and epigenetic studies in non-syndromic oral clefts. *Oral Dis*. 2021;28(5):1339-1350. doi:10.1111/odi.14146
7. Mossey P, Modell B. Epidemiology of oral clefts 2012: An international perspective. *Front Oral Biol Basel, Karger*. 2012;16:1-18. doi:10.1159/000337464
8. Notterman DA, Mitchell C. Epigenetics and understanding the impact of social determinants of health. *HHS Public Access*. 2015;62(5):1227-1240. doi:10.1016/j.pcl.2015.05.012.
9. Garland MA, Reynolds K, Zhou CJ. Environmental mechanisms of orofacial clefts. *Birth Defects Res*. 2020;112(19):1660-1698. doi:10.1002/bdr2.1830.
10. Messer LC, Luben TJ, Mendola P, Carozza SE, Horel SA, Langlois PH. Urban-Rural residence and the occurrence of cleft lip and cleft palate in Texas, 1999-2003. *Ann Epidemiol*. 2010;20(1):32-39. doi:10.1016/j.annepidem.2009.09.006.
11. Vieira AR, Furtado PGC, Lacerda Filho VV, et al. Orofacial cleft frequency differences depending on geographic origin: Coast vs. Inland. *Frontiers in Dental Medicine*. 2021;2(April):1-6. doi:10.3389/fdmed.2021.670948
12. Kapos FP, White LA, Schmidt KA, Hawes SE, Starr JR. Risk of non-syndromic orofacial clefts by maternal rural-urban residence and race/ethnicity: A population-based case-control study in Washington state 1989-2014. *Paediatr Perinat Epidemiol*. 2021;35(3):292-301. doi:10.1111/ppe.12727
13. , DataSUS - Tabnet. Accessed September 4, 2023. https://datasus.saude.gov.br/informacoes-de-saude-tabnet/.
14. , International Statistical Classification of Diseases and Related Health Problems - CID10. Accessed September 4, 2023. https://cid10.com.br.
15. , Instituto Brasileiro de Geografia e Estatística - IBGE. Municípios defrontantes com o mar. Accessed September 4, 2023. https://www.ibge.gov.br/geociencias/organizacao-do-territorio/estrutura-territorial/24072-municipios-defrontantes-com-o-mar.html?=&t=acesso-ao-produto.
16. Inchingolo AM, Fatone MC, Malcangi G, et al. Modifiable risk factors of non-syndromic orofacial clefts : A systematic review. *Children*. 2022;9(12):1846.
17. Vieira AR, Orioli IM, Murray JC. Maternal age and oral clefts: A reappraisal. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2002;94(5):530-535. doi:10.1067/moe.2002.128875
18. Medronho R de A, Bloch KV, Luiz RR, Werneck GL. *Epidemiologia. 2ª*. Editora Atheneu; 2009.
19. Oliveira Md, Andrade SdA, Dimech GS, et al. Avaliação do Sistema de Informações sobre Nascidos Vivos. Brasil, 2006 a 2010. *Epidemiologia e Serviços de Saúde*. 2015;24(4):629-640. doi:10.5123/s1679-49742015000400005.
20. Spina V, Psillakis JM, Lapa FS, Ferreira MC. Classificação das fissuras lábio-palatinas. *Rev Hosp Clin Fac Med*. 1972;27(1):5-6.
21. Mancilla VJ, Peeri NC, Silzer T, et al. Understanding the interplay between health disparities and epigenomics. *Front Genet*. 2020;11(August):1-14. doi:10.3389/fgene.2020.00903
22. Cruz LA. *Mecanismos Genéticos e Epigenéticos Na Etiologia Das Fissuras Orofaciais*. Instituto de Biociências da Universidade de São Paulo; 2017.

4.4 ARTIGO IV:

Manuscrito submetido no periódico *Maternal and Child Health Journal* (QUALIS CAPES A1) em Jul/2023.

OROFACIAL CLEFT AND POOR BIRTH HEALTH OUTCOMES: A POPULATIONAL CROSS-SECTIONAL STUDY

Abstract

Objective: to examine whether newborns with orofacial are at increased prevalence of poor birth health outcomes. **Methods:** This is a populational cross sectional study conducted with information from 18,684,399 live births born between 2017 to 2021, collected from the US Vital Statistics Natality Birth Data. The prevalence ratios were done between orofacial clefts status (syndromic or non syndromic) and each child's birth health variables (delivery method, maternal morbidity, Apgar 5, Apgar 10, gestational age, birth weight, abnormal conditions, infant breastfed at discharge) were calculated, assuming p value < 0.05 as statistically significant. **Results:** The prevalence ratios showed that newborns with orofacial clefts were more susceptible to born by cesarean section, have lower birth weight and lower Apgar scores, have shorter gestational age, experienced more abnormal conditions and more difficulty to be breastfed than newborns born without these conditions. These ratios were even higher among those with syndromic orofacial clefts. **Conclusion:** This study provides evidence that newborns with orofacial cleft are at increased prevalence of poor birth health outcomes.

Key-words: Orofacial cleft, Cleft Lip, Cleft palate, Perinatal Care.

Introduction

Orofacial clefts (OC) are the most common craniofacial congenital abnormally worldwide (Mossey et al., 2009). At the United States, they represent the second most common birth defect, only after Down Syndrome (Nasreddine et al., 2021). The clefts have an impact in children`s life and even after surgical repair, these children remain at increased risk for middle ear disease, velopharyngeal dysfunction, and malocclusion and require ongoing follow-up with a multidisciplinary team (Worley et al., 2018). Due to their prevalence and impact, OC constitute a serious health burden worldwide (Denadai & Lo, 2022).

OC occur due to a failure during the craniofacial embryologic development (Mossey et al., 2009). The majority of them are classified as nonsyndromic or isolated because they appear without any other structural or cognitive anomalies (Leslie, 2022). They are considered as having a multifactorial etiology since genetic and environmental risk factors play a role in their occurrence. In the other hand, OC are called as syndromic when they occur due to Mendelian inherence, which is generally related to a single gene but can be complicated by factors such as variable expressivity, incomplete penetrance, and the inherent difficulty in comprehensively phenotyping an individual at any given point in time (Leslie, 2022).

Due to the complexity of the etiology of OC, efforts have been done in order to identify genes and genetic variants for them (Khan et al., 2020; Leslie, 2022; Leslie & Marazita, 2013; Reynolds et al., 2020; Stuppia et al., 2011). Also, several factors have been studying as potential risk for OC, such as: maternal smoking, age, and nutrition (Kawalec et al., 2015; Lopes & Vieira, 2020; Vieira et al., 2002).

While most of the studies have been published in the areas of etiology and treatment of patients with OC, few studies were done focused on perinatal and outcomes (Wyszynski et al., 2003). Furthermore, perinatal conditions for both mothers and children have been less explored, in part because of the difficulty in obtaining reliable data from a large number of affected individuals (Wyszynski & Wu, 2002). In this context, this study aimed to examine whether newborns with orofacial clefts are at increased prevalence of poor birth health outcomes.

Methods

This is a populational cross sectional study conducted with data from the US Vital Statistics Natality Birth Data (VSNBD) for the last five years available (2017 to 2021). Natality Data, which are provided by National Vital Statistics System, showed demographic and health data for births occurring in the United States for each year. The microdata is based on information registered in the Standard Certificate of Live Birth, fully implemented in all States of the United States (US) and District of Columbia in 2016 (Center for Disease Control - CDC, n.d.). These vital statistics data are interpreted according to various qualifying factors and methods of classification in order to verify their value for most general purposes (Center for Disease Control - CDC, n.d.), making these data the most comprehensive and reliable US national source of birth information (Grünebaum et al., 2020).

This study used public-use natality data from an open access information system, in which the data are disbursed without identifying the subjects, waiving the need for approval Institutional Review Board. Investigators here had no contact with human subjects. Also, this study was reported according to the recommended STROBE (STrengthening the Reporting of Observational studies in Epidemiology) statement (Vandenbroucke JP, 2020).

In this study, the presence of OC was considered as the independent variable. Clefts were divided according to the presence or absence of other malformations or anomalies respectively as syndromic (SOC) or non syndromic (NSOC), which can be also called isolated clefs (Watkins et al., 2014). In order to minimize potential sources of bias, infants whose birth certificate did not indicate the presence of OC ("unknown or not stated") and those with positive or unknown diagnosis for any of other 11 congenital malformations (cyanotic congenital heart disease, hypospadias, Down syndrome, gastroschisis, suspected chromosomal disorder, meningomyelocele/spina bifida, congenital diaphragmatic hernia, limb reduction defect, anencephaly, and omphalocele) without OC combined were excluded. As dependent variables, it was considered child's birth health conditions (delivery method, maternal morbidity, Apgar 5, Apgar 10, gestational age, birthweight, abnormal conditions, infant breastfed at discharge) (Table 1).

Table 1 – Description and categorization of the dependent variables

Dependent variable	Description	Categories
Delivery method	Final route and method of delivery.	Cesarean; Vaginal.
Maternal morbidity	Occurrence of any of the conditions: maternal transfusion, perineal laceration, ruptured uterus, unplanned hysterectomy; admission to intensive care unit.	Present; Absent.
Apgar 5	Measure of the need for infant resuscitation at 5 minutes after delivery. It is a predictor of the infant's chances of surviving the first year of life. It ranges from 0 to 10. A score of 7 or greater indicates that the neonate is in good to excellent physical condition.	Below 7; 7 or more.
Apgar 10	Measure of the need for infant resuscitation at 10 minutes after delivery. It is a predictor of the infant's chances of surviving the first year of life. It ranges from 0 to 10. A score of 7 or greater indicates that the neonate is in good to excellent physical condition.	Below 7; 7 or more.
Gestational age	Births occurring before 37 completed weeks of gestation are considered to be preterm birth*.	Below 37 weeks; 37 weeks or more
Birthweight	Low birthweight is defined as less than 2,500 grams, and very low birthweight as less than 1,500 grams*.	Below 2,500g; 2,500g or more.
Abnormal conditions	Occurrence of any of the conditions: assisted ventilation required, admission to neonatal intensive care unit, newborn given surfactant replacement therapy, antibiotics received by the newborn for suspected neonatal sepsis, and seizure or serious neurological dysfunction.	Present; Absent.
Infant breastfed at discharge	Information regarding the infant capacity to be breastfed or not during the period from birth to discharge from the hospital.	Yes; No.

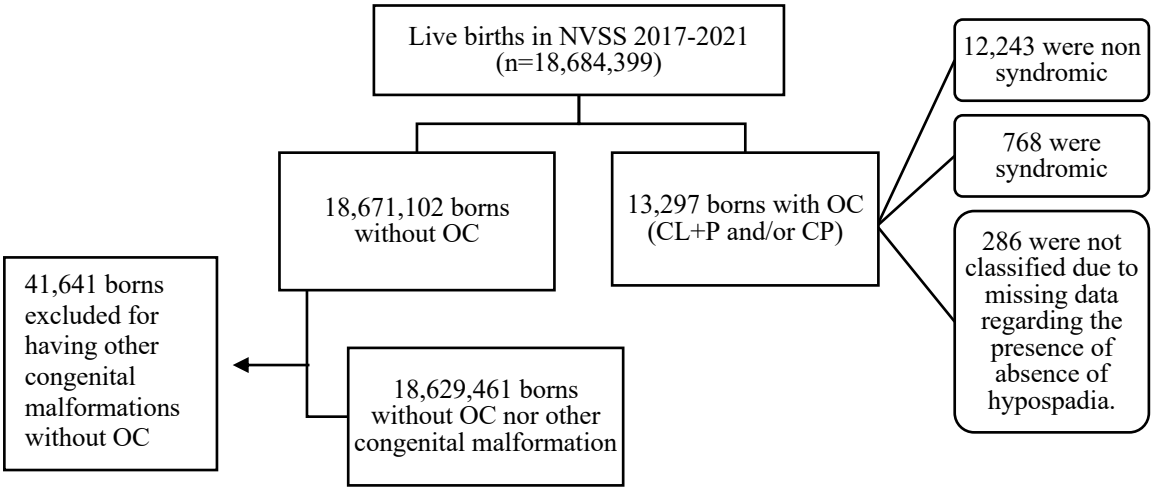
*Definitions according to ICD-10 (WORLD HEALTH ORGANIZATION, 1967)
Information based on the User Guide to the US Natality Public Use File

All variables were analyzed previously according to the criteria of incompleteness proposed by Romero and Cunha (Maia et al., 2017; Romero & Cunha, 2007) as excellent (< 5%), good (5% to 9.9%), regular (10% to 19.9%), poor (20% to 49.9%), and very poor (≥ 50%). Only those with excellent or good degree of incompleteness was studied, and the missing data was excluded from analyzes. Differences between those with and without OC were evaluated using chi-square. Prevalence ratios were calculated between OC status (overall, syndromic or non syndromic) and each child's birth health variables. All these analyzes were done using STATA (Software for Statistics and Data Science), assuming a p value ≤ 0.05 and 95% confidence intervals (95% C.I.) as statically significant.

Results

This populational cross sectional study analyzed data from 18,684,399 live births that were born between 2017 and 2021, according to the US Vital Statistics Natality Birth Data. The majority of the study participants are male (51%), with mothers were under 35 years (81%) and self-reported as white (73%). From those population, 13,297 (0.7%) were born with OC, resulting in a prevalence of 7.1/10.000 for the period. Among these cases, 12,243 were non syndromic, 768 were syndromic, and 286 (<1%) could not be classified due to missing data regarding the presence of absence of hypospadia. From the total live births, 41,641 were excluded from these analyses, since these children had other congenital malformations without OC (Figure 1). The dependent variables degree of completeness ranged from 91.75% (birth weight) to 99.98% (abnormal conditions), with the exception of infant breastfed at discharge that was filled in 87.5% of the records.

Figure 1 - Population characterization



OC: orofacial clefts; CL+P: cleft lip with or without palate; CP: cleft palate only

The prevalence ratios between orofacial clefts status (syndromic or non-syndromic) and each child's birth health outcome can be seen at the Table 2. Results showed that OC were associated with all dependent variables studied, meaning that children with OC experience poorer health outcomes at birth compared to those without clefts.

Table 2 - Prevalence ratios (PR) between orofacial clefts and poor child's birth health outcomes

	PR	p value	95%C.I.
Cesarean delivery method			
Orofacial clefts (all)	1.17	**0.000**	1.150243 - 1.20195
Syndromic Orofacial clefts	1.46	**0.000**	1.354827 - 1.576746
Non syndromic Orofacial clefts	1.15	**0.000**	1.12627 - 1.179935
Maternal morbidity			
Orofacial clefts (all)	1.32	**0.000**	1.171888 - 1.502079
Syndromic Orofacial clefts	1.03	0.913	0.5747038 - 1.858018
Non syndromic Orofacial clefts	1.29	**0.001**	1.134938 - 1.474892
Apgar 5 below 7			
Orofacial clefts (all)	4.41	**0.000**	4.183774 - 4.664718
Syndromic Orofacial clefts	22.83	**0.000**	21.1477 - 24.64634
Non syndromic Orofacial clefts	3.05	**0.000**	2.850266 - 3.273138
Apgar 10 below 7			
Orofacial clefts (all)	8.26	**0.000**	7.608557 - 8.978606
Syndromic Orofacial clefts	60.04	**0.000**	53.82406 - 66.98489
Non syndromic Orofacial clefts	4.58	**0.000**	4.074743 - 5.147144
Premature birth			
Orofacial clefts (all)	1.76	**0.000**	1.696943 - 1.825997
Syndromic Orofacial clefts	4.61	**0.000**	4.274793 - 4.976642
Non syndromic Orofacial clefts	1.53	**0.000**	1.470349 - 1.597562
Low birth weight			
Orofacial clefts (all)	2.20	**0.000**	2.120008 - 2.278996
Syndromic Orofacial clefts	6.69	**0.000**	6.282143 - 7.136014
Non syndromic Orofacial clefts	1.83	**0.000**	1.763017 - 1.917247
Presence of abnormal conditions			
Orofacial clefts (all)	3.75	**0.000**	3.679378 - 3.830107
Syndromic Orofacial clefts	5.56	**0.000**	5.267166 - 5.884141
Non syndromic Orofacial clefts	3.55	**0.000**	3.477751 - 3.63343
Infant cannot be breastfed at discharge			
Orofacial clefts (all)	2.06	**0.000**	2.01281 - 2.112209
Syndromic Orofacial clefts	3.44	**0.000**	3.2359 - 3.668455
Non syndromic Orofacial clefts	1.96	**0.000**	1.91343 - 2.015683

Bold indicates statistically significant p-values under the threshold 0.05
PR: Prevalence Ratio

Discussion

In this study, children with OC experienced poorer health outcomes at birth than those children without clefts. OC were statically associated with higher occurrence of cesarean delivery, maternal morbidity, premature births, abnormal conditions in the newborn, difficulty in being breastfed at discharge, low Apgar scores, and low birth weight. When analyzing by

the type of cleft, the associations were in general higher among those with the syndromic form of orofacial clefts.

The cesarean section consists of the most common surgery performed in the United States, with more than a million cesarean deliveries performed each year (Sung & Mahdy, 2023). Regarding middle-income and low- income countries, caesarean section rate continues to increase (Betrán et al., 2021). In this study, cesarean section had a discrete higher prevalence among children with clefts (PR=1.17, p=0.000, 95%C.I.=1.150-1.202). This result is in part expected since congenital abnormalities are one of the fetus indications for cesarean section (Barber et al., 2011; Sung & Mahdy, 2023) However, optimizing its use is of global concern due to its risk for potential complications related to maternal and perinatal mortality and morbidity (Betrán et al., 2021).

Maternal morbidity includes conditions such as: maternal needs for transfusion, perineal laceration, ruptured uterus, unplanned hysterectomy, and admission to Intensive Care (Center for Disease Control - CDC, n.d.). As like cesarean section, this outcome was discrete higher associated with OC (PR=1.32, p=0.000, 95%C.I.=1.172-1.502). However, when analyzed by the type of cleft, it was only associated with the non syndromic cases (PR=1.29, p=0.001, 95%C.I.= 1.135-1.475).

Apgar score is a test given to a baby immediately after birth, checking how well the newborn tolerates the environment outside the uterus. It was designed to help identify infants that require respiratory support or other resuscitative measures, not as an outcome (infants scoring less than 7) (Simon et al., 2023). Through this study, it was noticed that children with OC had a higher prevalence of lower Apgar scores for both at five and 10 minute (RP=4.41, p=0.000, 95%C.I.= 4.184-4.665; PR=8.26, p=0.000, 95%C.I.=7.609-8.979) than those without clefts. Previously study focused in nonsydromic clefts also found low Apgar scores more frequent among these newborns (Wyszynski & Wu, 2002). When analyzing only the syndromic cases, the associations ware even stronger, reflecting in an occurrence 20 times to 60 times higher of low Apgar scores, respectively at five and ten minutes.

The use of oxytocin, oxygen and resuscitation in the delivery room showed a significant association with Apgar<7 at five minutes of life (Saraiva et al., 2018). Additionally, abnormal conditions in the newborn consist in necessity of assisted ventilation, admission to Neonatal Intensive Care Unit, antibiotics, and development of surfactant or seizures (Center for Disease Control - CDC, n.d.). Children with OC were at almost four times higher risk of occurrence of abnormal conditions (RP=3.75, p=0.000, 95%C.I.=3.679-3.830). A case-control study using national data from United States found similar results for non syndromic clefts, demonstrating

that these newborns are at higher risk of requiring assisted ventilation, independent of their gestational age (Wyszynski & Wu, 2002).

Premature births occurred in about 13.4 million of the pregnancies in 2020 worldwide (World Health Organization, 2023). Data from our study revealed that OC are associated with premature births (OR=1.76, p=0.000, 95%C.I.=1.697-1.826). A study using nationwide hospital births data in United States also found an association between OC and prematurity (PR=1.83) (Shehan et al., 2021). When analyzing by the type of cleft, we found that non syndromic cases of clefts had 1.5 times higher (PR=1.53, p=0.000, 95%C.I.=1.763-1.917) occurrence of prematurity, and among syndromic cases this occurrence was almost five times higher (PR=4.61, p=0.000, 95%C.I.=4.275-4.977). A nationwide population-based study in South Korea found similar results, that both children with non-syndromic and syndromic clefts had higher risk of premature births compared (OR: non-syndromic 1.43, syndromic 5.29) (Ryu et al., 2022). Although the relationship between OC and prematurity has not been completely understand, these results help to stench the hypothesis that prematurity and maternal factors that contribute to prematurity are important risk factors for the development of clefts, helping to improve risk stratification, maternal counseling, and interventions (Shehan et al., 2021).

Birth weight is a strong predictor of future health outcomes and human capital attainment (Walker et al., 2007). Low birth weight in children with OC highlights a potential pathway for several of the aforementioned effects of cleft later in life. In this study, OC was associated with low birth weight. Children with OC had twice more low birth weight, being the association stronger for syndromic than nonsyndromic clefts (PR: nonsyndromic = 1.83, p=0.000, 95%C.I.=1.763 1.917; syndromic = 6.69, p=0.000, 95%C.I.= 6.282-7.136), with similar results found in the literature (DeRoo et al., 2003). One reason could be that affected pregnancies especially those with syndromic forms, known to be more severe, are complicated by more health problems and risk factors that reduce birth weight. Authors suggest that improving prenatal care access to pregnant women at-risk for OC or who receive a prenatal diagnosis of OC may play an important role in preventing lower birth weight (Nyarko et al., 2013).

Breastfeeding is associated with improved cognitive development and because of that, it should be supported by clinicians as a critical public health measure (Eidelman, 2013). OC result in feeding problems because the structural lip and/or palatal defects compromises the children's abilities of suction, swallow, and breath, necessary for feeding (Miller, 2011). As result, these children may experience malnourishment, low weight gain and growth, and learning disabilities (Beaumont, 2008). These evidences support our findings that children with

clefts had more difficulties in being breastfed at discharge (PR=2.06, p=0.000). The degree of feeding difficulty depends on the type and severity of the infant's cleft (Miller, 2011). Our result showed an association even stronger among children with syndromic clefts (PR=3.44, p=0.000). Authors recognized that support from healthcare professionals is critical for improve breastfeeding and the use of human milk in children with clefts (Burca et al., 2016).

The principal limitation of this study was the use of vital statistics data, which can be related to bias in the original records or in tabulation due to missing data. However, this limitation does not decrease the value of US Vital Statistics Natality Birth Data since they are analyzed by various qualifying factors in order to increase its reliability (Center for Disease Control - CDC, n.d.). These data are the only US database for births that is national in scope, and our analyzes revealed an excellent to good degree of information incompleteness, strengthening their use as a comprehensive and reliable US national source of birth data. Another limitation would be the identification of OC cases solely from birth certificates registry due to chances of imprecision in cleft registration. However, OC are a type of congenital anomaly likely to be observable at birth, with relatively well-defined categories. Also, the registration of the presence or absence of OC in the birth certificate is done after medical evaluation. These factors minimize chances of OC misclassification (i.e., the infants who did not have diagnosis of OC at birth but were diagnosed later in life were treated as not having OC in this study) and increase the sensitivity. Furthermore, several studies have been performed to study birth congenital anomalies (Block et al., 2013; Carmichael et al., 2014; Marengo et al., 2013; Wu et al., 2020; Yang et al., 2022).

Conclusions for practice

This study was intended to identify perinatal characteristics of mothers and newborns that could be related to a higher occurrence of both non syndromic and syndromic OC. In summary, it can be suggested that cesarean delivery, maternal morbidity, premature birth, abnormal conditions in the newborn, difficulty in being breastfed at discharge, low Apgar scores, and low birth weight are outcomes more frequently observed in pregnant mothers of offspring with OC than in the general population of pregnant women. This identification might assist public health systems in planning the allocation of resources in order to reduce the effects of OC in children.

References

Barber, E. L., Lundsberg, L. S., Belanger, K., Pettker, C. M., Funai, E. F., & Illuzzi, J. L. (2011). Indications contributing to the increasing cesarean delivery rate. *Obstetrics and Gynecology*, 118(1), 29–38. https://doi.org/10.1097/AOG.0b013e31821e5f65

Beaumont, D. (2008). A study into weight gain in infants with cleft lip/palate. *Paediatric Nursing*, 20(6), 20–23.

Betrán, A. P., Ye, J., Moller, A. B., Souza, J. P., & Zhang, J. (2021). Trends and projections of caesarean section rates: Global and regional estimates. *BMJ Global Health*, 6(6), 1–8. https://doi.org/10.1136/bmjgh-2021-005671

Block, S. R., Watkins, S. M., Salemi, J. L., Rutkowski, R., Tanner, J. P., Correia, J. A., & Kirby, R. S. (2013). Maternal pre-pregnancy body mass index and risk of selected birth defects: Evidence of a dose-response relationship. *Paediatric and Perinatal Epidemiology*, 27(6), 521–531. https://doi.org/10.1111/ppe.12084

Burca, N. D. L., Gephart, S. M., Miller, C., & Cote, C. (2016). Promoting breast milk nutrition in infants with cleft lip and/or palate. *Advances in Neonatal Care*, 16(5), 337–344. https://doi.org/10.1097/ANC.0000000000000305

Carmichael, S. L., Ma, C., Tinker, S. C., Rasmussen, S. A., Shaw, G. M., & National Birth Defects Prevention Study. (2014). Maternal stressors and social support as risks for delivering babies with structural birth defects. *HHS Public Access*, 28(4), 338–344. https://doi.org/10.1111/ppe.12123.Maternal

Center for Disease Control - CDC. (n.d.). User Guide to the 2021 Natality Public Use File.

Denadai, R., & Lo, LJ. (2022). The Burden of Orofacial Clefts: Global Trends and Focus on Underresourced Regions. In Current Concept in Cleft Surgery (pp. 13–20). *Springer*. https://doi.org/doi.org/10.1007/978-981-19-3163-5_2

DeRoo, L. A., Gaudino, J. A., & Edmonds, L. D. (2003). Orofacial cleft malformations: Associations with maternal and infant characteristics in Washington State. *Birth Defects Research Part A - Clinical and Molecular Teratology*, 67(9), 637–642. https://doi.org/10.1002/bdra.10114

Eidelman, A. I. (2013). Breastfeeding and cognitive development: Is there an association? *Jornal de Pediatria*, 89(4), 327–329. https://doi.org/10.1016/j.jped.2013.05.002

Grünebaum, A., McCullough, L. B., Orosz, B., & Chervenak, F. A. (2020). Neonatal mortality in the United States is related to location of birth (hospital versus home) rather than the type of birth attendant. *American Journal of Obstetrics and Gynecology*, 223(2), 254.e1-254.e8. https://doi.org/10.1016/j.ajog.2020.01.045

Kawalec, A., Nelke, K., Pawlas, K., & Gerber, H. (2015). Risk factors involved in orofacial cleft predisposition-review. *Open Medicine (Poland)*, 10(1), 163–175. https://doi.org/10.1515/med-2015-0027

Khan, A. M. I., Prashanth, C., & Srinath, N. (2020). Genetic etiology of cleft lip and cleft palate. AIMS *Molecular Science*, 7(4), 328–348. https://doi.org/10.3934/molsci.2020016

Leslie, E. J. (2022). Genetic models and approaches to study orofacial clefts. *Oral Diseases*, 28(5), 1327–1338. https://doi.org/10.1111/odi.14109

Leslie, E. J., & Marazita, M. L. (2013). Genetics of cleft lip and cleft palate. *American Journal of Medical Genetics, Part C: Seminars in Medical Genetics*, 163(4), 246–258. https://doi.org/10.1002/ajmg.c.31381

Lopes, F., & Vieira, M. G. (2020). Fatores de risco ambientais e teratogênicos associados às malformações congênitas: um estado do conhecimento Fatores de risco ambientais e teratogênicos associados às malformações congênitas: um estado do conhecimento. *RELACult*, 6, 1–24.

Maia, L. T. de S., Souza, W. V. de, Mendes, A. da C. G., & Silva, A. G. S. da. (2017). Use of linkage to improve the completeness of the SIM and SINASC in the Brazilian capitals. *Rev. Saúde Pública*, 51, 112.

Marengo, L., Farag, N. H., & Canfield, M. (2013). Body mass index and birth defects: Texas, 2005-2008. *Maternal and Child Health Journal*, 17(10), 1898–1907. https://doi.org/10.1007/s10995-012-1214-5

Miller, C. K. (2011). Feeding issues and interventions in infants and children with clefts and craniofacial syndromes. *Seminars in Speech and Language*, 32(2), 115–126. https://doi.org/10.1055/s-0031-1277714

Mossey, P. A., Little, J., Munger, R. G., Dixon, M. J., & Shaw, W. C. (2009). Cleft lip and palate. The Lancet, 374(9703), 1773–1785. https://doi.org/10.1016/S0140-6736(09)60695-4

Nasreddine, G., Hajj, J. El, & Ghassibe-sabbagh, M. (2021). Orofacial clefts embryology, classification, epidemiology, and genetics. *Mutation Research*, 787, 108373. https://doi.org/10.1016/j.mrrev.2021.108373

Nyarko, K. A., Lopez-Camelo, J., Castilla, E. E., & Wehby, G. L. (2013). Does the relationship between prenatal care and birth weight vary by oral clefts? Evidence using South American and united states samples. *Journal of Pediatrics*, 162(1). https://doi.org/10.1016/j.jpeds.2012.06.040

Reynolds, K., Zhang, S., Sun, B., Garland, M. A., Ji, Y., & Zhou, C. J. (2020). Genetics and signaling mechanisms of orofacial clefts. *Birth Defects Research*, 112(19), 1588–1634. https://doi.org/10.1002/bdr2.1754

Romero, D. E., & Cunha, C. B. da. (2007). Avaliação da qualidade das variáveis epidemiológicas e demográfi cas do Sistema de Informações sobre Nascidos Vivos, 2002. *Cad. Saúde Públic*, 23(3), 701–714.

Ryu, J. Y., Park, T. H., Cho, B. C., & Choi, K. Y. (2022). The prevalence, risk of premature births, mortality and causes of death of cleft lip with or without palate in South Korea: a nationwide population-based cohort study. *International Journal of Epidemiology*, 51(3), 974–983. https://doi.org/10.1093/ije/dyac019

Saraiva, J. P., Vogt, S. E., Rocha, J. D. S., Duarte, E. D., & Simão, D. A. D. S. (2018). Association between maternal and neonatal factors and Apgar in usual risk neonates. *Revista Da Rede de Enfermagem Do Nordeste*, 19, 3179. https://doi.org/10.15253/2175-6783.2018193179

Shehan, J. N., Danis, D. O., Bains, A., Basa, K., Marston, A. P., & Levi, J. R. (2021). Cleft Palate in Newborns Diagnosed With Prematurity. *Otolaryngology - Head and Neck Surgery (United States)*, 165(6), 887–894. https://doi.org/10.1177/01945998211002148

Simon, L., Hashmi, M., & Bragg, B. (2023). Apgar Score. In StatPearls Publishing.

Stuppia, L., Capogreco, M., Marzo, G., La Rovere, D., Antonucci, I., Gatta, V., Palka, G., Mortellaro, C., & Tetè, S. (2011). Genetics of syndromic and nonsyndromic cleft lip and palate. *Journal of Craniofacial Surgery*, 22(5), 1722–1726. https://doi.org/10.1097/SCS.0b013e31822e5e4d

Sung, S., & Mahdy, H. (2023). Cesarean Section. In StatPearls Publishing. Treasure Island.

Vandenbroucke JP, von E. E. A. D. G. P. M. C. P. S. P. C. S. J. and E. M. (2020). Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. *In Int J Surg* 2014; 12: 1500-1524 (Vol. 12, pp. 1500–1524).

Vieira, A. R., Orioli, I. M., & Murray, J. C. (2002). Maternal age and oral clefts: A reappraisal. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics*, 94(5), 530–535. https://doi.org/10.1067/moe.2002.128875

Walker, S. P., Wachs, T. D., Gardner, J. M., Lozoff, B., Wasserman, G. A., Pollitt, E., Carter, J. A., & International Child Development Steering Group. (2007). Child development: risk factors for adverse outcomes in developing countries. *The Lancet*, 369(9557), 229–242. https://doi.org/10.1136/bmjopen-2017-018916

Watkins, S. E., Meyer, R. E., Strauss, R. P., & Aylsworth, A. S. (2014). Classification, Epidemiology, and Genetics of Orofacial Clefts. *Clinics in Plastic Surgery*, 41(2), 149–163. https://doi.org/10.1016/j.cps.2013.12.003

World Health Organization. (2023). Preterm birth. *Preterm Birth*. https://www.who.int/news-room/fact-sheets/detail/preterm-birth

World Health Organization (WHO). (1967). Manual of the International Statistical Classification of Diseases, Injuries, and Causes of Death (Vol. 1).

Worley, M. L., Patel, K. G., & Kilpatrick, L. A. (2018). Cleft Lip and Palate. *Clinics in Perinatology*, 45(4), 661–678. https://doi.org/10.1016/j.clp.2018.07.006

Wu, Y., Liu, B., Sun, Y., Du, Y., Santillan, M. K., Santillan, D. A., Snetselaar, L. G., & Bao, W. (2020). Association of maternal prepregnancy diabetes and gestational diabetes mellitus with congenital anomalies of the newborn. *Diabetes Care*, 43(12), 2983–2990. https://doi.org/10.2337/dc20-0261

Wyszynski, D. F., Sarkozi, A., Vargha, P., & Czeizel, A. E. (2003). Birth weight and gestational age of newborns with cleft lip with or without cleft palate and with isolated cleft palate. *The Journal of Clinical Pediatric Dentistry*, 27(2), 185–190. https://doi.org/10.17796/jcpd.27.2.475367q2601u3x4w

Wyszynski, D. F., & Wu, M. H. S. (2002). Prenatal and Perinatal Factors Associated With Isolated Oral Clefting. *Cleft Palate-Craniofacial Journal*, 39(33).

Yang, L., Wang, H., Yang, L., Zhao, M., Guo, Y., Bovet, P., & Xi, B. (2022). Maternal cigarette smoking before or during pregnancy increases the risk of birth congenital anomalies: a population-based retrospective cohort study of 12 million mother-infant pairs. *BMC Medicine*, 20(1). https://doi.org/10.1186/s12916-021-02196-x

4.5 ARTIGO V:

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COMPARATIVE STUDY OF INDIVIDUALS BORN WITH OROFACIAL CLEFTS IN BRAZIL AND UNITED STATES

Abstract

Purpose: To characterize the burden of orofacial clefts in Brazil by drawing comparisons in the prevalence, risk factors, and child's birth health conditions between those born with clefts in Brazil and the United States. **Methods:** Populational cross sectional study conducted with national data from 13,297 and 8,853 individuals born with orofacial cleft in Brazil and United States, respectively. Risk factors and child's birth health conditions variables were gathered. The prevalence of clefts in both countries and prevalence ratios (PR) were calculated. **Results:** The prevalence of clefts was 7.1/10.000 and 6.2/10.000 in United States and Brazil, respectively. Newborns born with clefts in Brazil were significantly more likely to have cleft palate (PR=1.32, p=0.000), prematurity (PR=1.37, p=0.000), born by cesarean (PR=0.000) and born with low birth weight (PR=1.49, p=0.000), their mothers tended to be older (PR=1.09, p=0.001), non-white (PR=0.48), and done less than 10 pre natal care appointments (PR=2.14, p=0.000), compared to the United States cohort. **Conclusion:** Frequency of risk factors and outcomes related to be born with clefts were more expressive for the Brazilian cohort, suggesting the role of the social determinants of health in increase individual's susceptibility to risk factors for clefts and poor child's birth health conditions.

Key-words: Orofacial cleft, newborn, pregnancy outcome.

Introduction

Orofacial clefts (OC) are congenital malformations characterized by an incomplete separation between the nasal and oral cavities during embryonic development[1–3]. The precise identification of the OC phenotype is crucial to understanding both the epidemiology and the etiology of this malformation4. Based on this, some classifications for OC have been proposed. The most basic one is based on the embryological origin and divides them into cleft lip with or without palate (CL+P) and cleft palate only (CP)[5]. They can also be classified based on the presence or absence of another anomaly as syndromic (SOC) or non-syndromic orofacial clefts (NSOC)[1,5,6].

Several risk factors play a role in the etiology of clefts[6–8]. Among those, there are biological factors (genetics, epigenetics, sex, ethnicity and maternal age)[7], and environmental exposures (tobacco and alcohol habits, nutritional deficiencies, drugs, maternal comorbidities, and others)[7,9–11]. It is hypothesized that social determinants of health (non-medical factors that influence health outcomes)[12] can increase individual's susceptibility to risk factors through various biological mechanisms, leading to clefts[10,13–18]. In some cases, the embryologic process can be perturbed by environmental factors that alter gene expression due to genetic or epigenetic modifications leading to OC while, in order cases, biologic conditions can adversely affect orofacial development[10].

OC is considered one of the most common craniofacial anomalies worldwide[1,2,19], affecting over 10 million lives, with an estimation of one new case every three minutes[4]. The global prevalence of OC is 1 in 700 live births[5,6], with geographical, racial, and sex variations5. OC constitutes a serious burden worldwide[4,20–23], not only due to its prevalence but also to its consequences in short and long term. Individuals with OC experience more pregnancy complications that can influence child's birth health conditions such as low gestational age, low birth weight, and low Apgar score[24]. They also present more health disabilities throughout the course of life, requiring long-term, sequential, and costly therapy[25].

In Brazil, although some studies have been done regarding risk factors for cleft[26–28], little attention has been focused on the pregnancy complications and child's birth health conditions related to OC. In the other hand, studies in United States have been starting to attempt to this issue[24,29]. The understatement of differences in risk factors as well as in OC child's birth health conditions between countries with different socioeconomic and living conditions may provide new insights for outlining preventive measures and gaps in maternal and child health care[24,30,31], that could help to minimize the burden of OC especially in middle-

low-income countries. Therefore, the objective of this study was to characterize the burden of orofacial clefts in Brazil by drawing comparisons in the prevalence, risk factors, and child's birth health conditions between those born with clefts in Brazil and in the United States.

Methods

This is a populational cross sectional study conducted with national data from two countries (United States Vital Statistics Natality Birth Data - VSNBD and Brazilian Live Birth Information System - SINASC) for the last five years available (2017 to 2021). This study used public-use birth data from open access information systems, in which the data are presented without identifying the subjects. Investigators here had no contact with human subjects. Also, this study was reported according to the recommended STROBE (STrengthening the Reporting of Observational studies in Epidemiology) statement[32].

VSNBD data are provided by National Vital Statistics System. It shows demographic and health data for births occurring in the United States for each year. The microdata is based on information registered in the Standard Certificate of Live Birth, fully implemented in all States of the United States and District of Columbia in 2016[33]. These vital statistics data are interpreted according to various qualifying factors and methods of classification in order to verify their value for most general purposes[33], making these data the most comprehensive and reliable US national source of birth information[34].

SINASC was officially implanted in 1990 by Brazilian Health Surveillance Department. It has the objective to collect data on births reported throughout the Brazilian territory and to provide data on birth rates to all Health System levels. These data make possible to build useful indicators for planning health services. SINASC is also based on information registered in the Standard Certificate of Live Birth[35]. Previous evidence supports the representativeness of SINASC for the Brazilian population[36].

Clefts were divided as CP and CL+P. In addition, they were classified according to the presence or absence of other malformations/anomalies respectively as SOC or NSOC[37]. Infants whose birth certificate did not indicate the presence of OC ("unknown or not stated") and those with positive or unknown diagnosis for any of other congenital malformations without OC combined were excluded. Live births that were coded for both Cleft Lip and Cleft Palate simultaneously were considered as having CL+P.

Risk factors (maternal ethnicity, maternal age, and sex of the infant) and child's birth health conditions (number of prenatal care appointments, delivery method, Apgar 5, gestational

age, birth weight) were gathered, taking into account their availability in both datasets (Table 1). All variables were analyzed previously according to the criteria of incompleteness proposed by Romero and Cunha[38] ranging from excellent (< 5%) to good (5% to 9.9%) degree of completeness.

Table 1 – Description and categorization of the dependent variables

	Variable	Description*	Categories
Risk factors	Maternal age	Number of completed years of the parturient at the time of delivery.	Below 35 years; 35 years or more.
	Maternal ethnicity	Corresponding category self-declared by mother.	Non-white; White
	Sex of the infant	Categories corresponding to the sex of the newborn.	Female; Male.
Child's birth health conditions	Delivery method	Final route and method of delivery.	Cesarean; Vaginal.
	Number of prenatal care appointments	Number of prenatal appointments	Below 10; 10 or more.
	Apgar 5	Measure of the need for infant resuscitation at 5 minutes after delivery. It is a predictor of the infant's chances of surviving the first year of life. It ranges from 0 to 10. A score of 7 or greater indicates that the neonate is in good to excellent physical condition.	Below 7; 7 or more.
	Gestational age	Number of weeks of gestation. Births occurring before 37 completed weeks of gestation are considered to be preterm birth.	Below 37 weeks; 37 weeks or more.
	Birthweight	Low birthweight is defined as less than 2,500 grams, and very low birthweight as less than 1,500 grams.	Below 2,500g; 2,500g or more.

*Information based on the User Guide to the US and Brazilian Natality Public data[33,35]

The prevalence of OC in both countries was calculated as: number of live births with OC divided by the total number of live births in the same period and location, multiplied by 10,000. All variables are analyzed as categorical data. Differences in variables between those with OC in Brazil and in the United States (US) were evaluated using chi-square. Prevalence ratios (PR) were calculated to compare the occurrence risk of each variable in those with OC between both countries. All these analyzes were done using R Software, assuming a p value $\leq$ 0.05 and 95% confidence interval (95% C.I.) as statically significant.

Results

This populational cross section study analyzed data from children with OC that were born between 2017 and 2021, according to Birth Vital Statistics from US and Brazil. For the US population, 13,297 children born with OC, resulting in a prevalence of 7.1/10.000 for the

period. Regarding Brazilian population, 8,853 children born with OC and the resulted prevalence was 6.2/10.000 for the period.

Distribution of OC by type and by the analyzed variables for both countries, with their PR is presented in Table 2. NSOC and CL+P were the most frequent type and phenotype of cleft in both countries, respectively. However, the occurrence of CP was 32% higher (PR=1.32, p=0.000) in Brazil. For both countries, most of those born with OC were male, without statistically significant differences. The Brazilian cohort was composed primarily of non-white mothers, whereas the US cohort was mostly white (PR=0.48). Also, Brazilian mothers tended to be older (PR=1.09, p=0.001) and frequent less prenatal care appointments (PR=2.14, p=0.000), comparing to United States. The occurrence of premature birth (less than 37 weeks of gestation) (PR=1.37, p=0.000), cesarean (PR=0.000, p=1.69) and with low birth weight (PR=1.49, p=0.000) was higher in Brazilian cohort of cleft individuals.

Table 2 - Distribution of orofacial cleft by the characteristics analyzed for both countries with their prevalence ratios (PR)

	Orofacial cleft (Brazil)		Orofacial cleft (United States)			
	(n)	(%)	(n)	(%)	p value	PR (95%C.I.)
Cleft type					**0.000**	
CL/P	5,603	63%	9,596	72%		ref
CP	3,250	37%	3,701	28%		**1.32 (1.27-1.37)**
Cleft classification					NaN	
Non syndromic	6,175	70%	12,243	94%		ref
Syndromic	2,678	30%	768	6%		**5.12 (4.75-5.52)**
Maternal age					**0.001**	
< 35 years	7,042	79%	10,811	81%		ref
≥ 35 years	1,811	21%	2,486	19%		**1.09 (1.03-1.15)**
Maternal ethnicity					NaN	
Non-white	5,261	61%	2,695	20%		ref
White	3,332	39%	10,602	80%		**0.48 (0.47-0.5)**
Sex of infant					0.694	
Female	3,779	43%	5,698	43%		ref
Male	4,985	57%	7,599	57%		1.00 (0.97-1.03)
Number of prenatal care appointments					**0.000**	
≥ 10 visits	2,829	32%	9,097	68%		ref
< 10 visits	5,959	68%	4,200	32%		**2.14 (2.08-2.21)**
Delivery method					**0.000**	
Vaginal	3,266	37%	8,154	63%		ref
Cesarean	5,571	63%	4,849	37%		**1.69 (1.64-1.74)**
Apgar 5					0.129	
≥ 7	7,956	91%	11,902	91,5%		ref
<7	798	9%	1,109	8,5%		1.06 (0.98-1.17)

Gestational age					**0.000**	
≥37 weeks	6,705	76%	10,753	83%		ref
<37 weeks	2,080	24%	2,245	17%		**1.37 (1.3-1.45)**
Birth weight					**0.000**	
≥2,500g	6,539	74%	10,731	82%		ref
<2,500g	2,313	26%	2,280	18%		**1.49 (1.41-1.57)**

Bold indicates statistically significant p-values under the threshold 0.05 and 95% confidence interval.
NaN = Not a number

Discussion

OC are considered as one of most common craniofacial congenital anomalies[39]. This study found that the overall prevalence of OC was higher in US than in Brazil. Potential OC risk factors and outcomes identified in the Brazilian population included maternal age, prenatal care, delivery method and prematurity, contributing to the burden of OC in the country. Our finds suggest the role of the social determinants of health in increase individual's susceptibility to risk factors for clefts and poor child's birth health conditions.

The prevalence of OC varies across nations and regions, influenced by factors such as ethnicity, demographics, climatic conditions, cultural diversity, and variations in prenatal care programs[40]. US seems to have a higher prevalence of cleft compared to Brazil, as evidenced by other studies. A previous national populational study at US for 2010-2014 found it was the third most common birth defect, occurring in approximately 10/10,000 live births[41]. In Brazil, prevalence of cleft reported through a recent national populational study was 5.2/10,000[28].

The expected prevalence of NSOC is about 70%[42], as it closely happened in Brazil. For US, the occurrence NSOC was greater than the expect (94%), which could be related to a better genetic counseling practice that may decrease the occurrence of SOC over time, contributing to a proportional increase in the percentage of NSOC in the country[43]. Regarding the phenotype of cleft, both cohorts had more cases of CL+P than CP, as reporting in previous epidemiologic studies[28,39,44]. The frequency of CL+P was even more expressive in the United States (37% vs. 28%, p= 0.000), representing occurrence of 32% higher of CP (PR=1.32, p=0.000) in Brazil compared to US.

Advanced maternal age has been related to an increased risk for OC[45,46]. It can induce epigenetic changes during the fetal development, leading to stillbirth and fetal abnormalities[47]. In our study, most of the mother were young, as previously reported[30]. When analyzing only mother with 35 or more, our data showed a higher frequency of advanced maternal age among children with OC from Brazilian population (21% vs. 19%, p=0.001), meaning an occurrence 9% higher of OC related to older mothers in this country (PR=1.09, p=0.001).

Maternal white ethnicity was related with a higher occurrence of clefts in US compared to Brazil (80% vs. 29%). For the United States population, our results align with the literature that has shown that Black/African Brazil American populations are affected with OC at lower rates than white, Asian, and Latin American populations[39]. Previous literature showed lower cleft prevalence rates in African-derived populations in the United States[18]. In Brazil, due to the effect of racial miscegenation, it is difficult to understand the role ethnicity in the occurrence of OC. Some studies[48–51] in this country reported more OC in non-white race. A possible explanation would be related to social inequality, most presented in low-income social strata, which could decrease access to the health system, predisposing to more OC in this population. Also, Brazilian population has highly variable genomic ancestry[52], and ethnic miscegenation in this population could influence genetic variants on the susceptibility of NSOC[53,54].

The countries did not show differences in the occurrence of clefts regarding the sex of infant (p=0.694). In both, there were a higher occurrence of clefts in male infants, as reported previously[28,30]. Also, studies report a commonly occurrence of CL+P in males and CP is in females[55–58].

Advancements in antenatal imaging techniques have facilitated the prenatal identification of OC, and it had been shown to play a pivotal role in preparing families, subsequently enhancing the quality of postnatal care and overall quality of life[59]. In addition, receiving prenatal care in the first trimester decreased the risk of cleft lip with or without cleft palate, probable due to the prevention of vitamin and mineral deficiencies and identification of harmful exposures that may disrupt embryonic orofacial development[18]. In the US, it seems that mothers had more prenatal care appointments during their pregnancy of OC child in US (68%) than in Brazil (32%). However, it could be related to the number of appointments took as reference for analysis since in Brazil the minimum number of appointments recommended is six[60] while in the United States this number is 15 appointments. Results have shown that a minimum frequency of six prenatal care visits was associated with lower rates of neonatal and adult Intensive Center Units admission and newborn mortality within the first 72 hours of life in Brazil[61].

Vaginal delivery can be attempted in most of the cases of congenital malformations, decreasing risks to the pregnant woman[62]. Our data showed that the number of vaginal deliveries in children with clefts was higher in US (63% vs. 37%, p=0.000). The election for cesarean delivery should be chosen taking into account the outcome benefits for the malformed fetus against potential maternal risks[62]. The occurrence cesarean among born with clefts in

Brazil was 69% higher compared to US (PR=1.69, p=0.000). This finding may be associated with the fact that Brazil has a high number of cesarean deliveries, being the second country in the rate of cesarean section worldwide (55.7%)[63].

Approximately 15 million newborns worldwide are born prematurely each year, and its related complications are the primary cause of death among children under the age of five[64]. There is a significantly elevated risk of OC in very low birth weight[65], which could be related to aneuploidy and single-gene disorders due to intrauterine growth restriction6. Furthermore, there is an inverse relationship between gestational age and the risk of increased mortality, morbidity, and the level of neonatal care required at birth[66]. A recent study 46 found that preterm newborns are more likely to develop OC. Considering the frequencies of gestational age (p=0.000) and low birth weight (p=0.000) in the countries studied here, it can be inferred the risk of a child with OC born prematurity was higher in Brazil than US. This result could be related to the role of the socio determinants of health in the occurrence of OC and prematurity, especially in lower-middle-income countries[67,68]. Factors such as low socioeconomic conditions could have contributed to increase vulnerability in pregnant women[17], predisposing to more prematurity in children with OC born in Brazil.

This study has some limitations inherent to the design of the case-control study, such as the convenience sample and the retrospective measurement of the predictive variables (susceptible to memory bias)[69]. In addition, the NAN in p values for the variables cleft classification and ethnicity represents a possible error in calculating the standard error of these variables that can be further adjusted. It does not imply missing of significance in the prevalence ratio since the 95% C.I. for the associations stills significant.

Belong those, the principal limitation here would be the use of vital statistics data, which is susceptible to bias in the original records or in tabulation due to missing or misclassifications. However, this limitation does not decrease the value of the national dataset used since they are analyzed by various qualifying factors in order to increase its reliability[33,70]. Another limitation would be the identification of OC cases solely from birth certificates registry due to chances of imprecision in cleft registration. However, OC are a type of congenital anomaly likely to be observable at birth, with relatively well-defined categories. Also, the registration of the presence or absence of OC in the birth certificate is done after medical evaluation. These factors minimize chances of OC misclassification (i.e., the infants who did not have diagnosis of OC at birth but were diagnosed later in life were treated as not having OC in this study) and increase the sensitivity. Furthermore, several studies have been

performed using this approach to select cases and assess the association between some risk factors and birth congenital anomalies and in the US[71–75] and in Brazil[49,76–78].

Conclusion

This study found that the overall prevalence of OC was higher in the United States than in Brazil. However, potential risk factors and outcomes related to be born with clefts (maternal age, maternal ethnicity, prenatal care, delivery method and prematurity) were more expressive for the Brazilian cohort, suggesting the role of the social determinants of health in increase individual's susceptibility to risk factors for clefts and poor child's birth health conditions.

References

1. Nasreddine G, El Hajj J, Ghassibe-Sabbagh M. Orofacial clefts embryology, classification, epidemiology, and genetics. *Mutat Res*. 2021;787:108373. doi:10.1016/j.mrrev.2021.108373
2. Marazita ML, Mooney MP. Current concepts in the embryology and genetics of cleft lip and cleft palate. *Clin Plast Surg*. 2004;31(2):125-140. doi:10.1016/S0094-1298(03)00138-X
3. Leslie EJ, Marazita ML. Genetics of cleft lip and cleft palate. *Am J Med Genet C Semin Med Genet*. 2013;163(4):246-258. doi:10.1002/ajmg.c.31381
4. Sandy J, Davies A, Humphries K, Ireland T, Wren Y. Cleft lip and palate: Care configuration, national registration, and research strategies. *J World Fed Orthod*. 2020;9(January):S40-S44. doi:10.1016/j.ejwf.2020.09.003
5. Mossey PA, Little J, Munger RG, Dixon MJ, Shaw WC. Cleft lip and palate. *The Lancet*. 2009;374:1773-1785. doi:10.1016/S0140-6736(09)60695-4
6. Dixon MJ, Marazita ML, Beaty TH, Murray JC. Cleft lip and palate: synthesizing genetic and environmental influences. *NIH Public Access*. 2011;3(3):203. doi:10.1038/nrg2933.Cleft
7. Inchingolo AM, Fatone MC, Malcangi G, et al. Modifiable Risk Factors of Non-Syndromic Orofacial Clefts : A Systematic Review. *Children*. 2022;9(12):1846.
8. Vieira AR. *Genetic and Environmental Factors in Human Cleft Lip and Palate*. Vol 16.; 2012.

9. Eshete M, Butali A, Abate F, et al. The Role of Environmental Factors in the Etiology of Nonsyndromic Orofacial Clefts. *Journal of Craniofacial Surgery*. 2020;31(1):113-116. doi:10.1097/SCS.0000000000005924
10. Garland MA, Reynolds K, Zhou CJ. Environmental mechanisms of orofacial clefts. *Birth Defects Res*. Published online 2020:1-39. doi:10.1002/bdr2.1830
11. Da Silva AM, De Lavôr JR, Freitas VS, Vieira AR. Risk of orofacial clefts in relation to maternal body mass index, diabetes and hypertension. *J Neonatal Perinatal Med*. Published online January 20, 2024:1-8. doi:10.3233/NPM-230118
12. Pan American Health Association(PAHO), World Health Organization (WHO), World HealthOrganization. Social Determinants of Health. Published 2022. Accessed January 15, 2022. https://www.paho.org/en/topics/social-determinants-health#:~:text=The Social Determinants of Health,the conditions of daily life".
13. Notterman DA, Mitchell C. Epigenetics and Understanding the Impact of Social Determinants of Health. *HHS Public Access*. 2015;62(5):1227-1240. doi:doi:10.1016/j.pcl.2015.05.012.
14. Smillie I, Yong K, Harris K, Wynne DM, Russell CJH. Socioeconomic influence on orofacial cleft patient care. Published online 2015:1-5. doi:10.1177/0036933014564133
15. Carmichael SL, Ma C, Shaw GM. socioeconomic measures, orofacial clefts, and conotruncal heart defects in California. *Birth Defects Res A Clin Mol Teratol*. 2009;85(10):850-857. doi:10.1002/bdra.20614
16. Clark JD, Mossey PA, Sharp L, Little J. Socioeconomic status and orofacial clefts in Scotland, 1989 to 1998. *Cleft Palate-Craniofacial Journal*. 2003;40(5):481-485. doi:10.1597/1545-1569(2003)040<0481:SSAOCI>2.0.CO;2
17. Nehra RA, Riasat Nahra A, Gulzar A, Ul Mobeen N, Riasat H, Zafar A. How Low Socioeconomic Status during Pregnancy Causes Orofacial Clefts in Newborns. *P J M H S*. 2021;15(2):432-433.
18. Vu GH, Warden C, Zimmerman CE, et al. Poverty and Risk of Cleft Lip and Palate: An Analysis of United States Birth Data. *Plast Reconstr Surg*. 2022;149(1):169-182. doi:10.1097/PRS.0000000000008636
19. Nagappan N, John J. Sociodemographic profile of orofacial cleft patients in India: A hospital-based study. *Int J Med Public Health*. 2015;5(1):35. doi:10.4103/2230-8598.151253

20. Agbenorku P. Orofacial Clefts: A Worldwide Review of the Problem. *ISRN Plastic Surgery*. 2013;2013:1-7. doi:10.5402/2013/348465
21. Assis IO, Lacerda RHW, G.N.Cavalcante B, Bezamat M, Modesto A, Vieira AR. IRE1 Less Common Homozygous Genotype in Families with Positive History of Cancer and Individuals Born with Cleft Lip and Palate. *Journal of Craniofacial Surgery*. Published online 2020:82.
22. Machado RA, de Toledo IP, Martelli-Júnior H, Reis SR, Guerra ENS, Coletta R Della. Potential genetic markers for nonsyndromic oral clefts in the Brazilian population: A systematic review and meta-analysis. *Birth Defects Res*. 2018;110(10):827-839. doi:10.1002/bdr2.1208
23. Denadai R, Lo LJ. The Burden of Orofacial Clefts: Global Trends and Focus on Underresourced Regions. In: *Current Concept in Cleft Surgery*. Springer; 2022:13-20. doi:doi.org/10.1007/978-981-19-3163-5_2
24. Wyszynski DF, Wu MHS. Prenatal and Perinatal Factors Associated With Isolated Oral Clefting. *Cleft Palate-Craniofacial Journal*. 2002;39(33).
25. Pittsburgh Orofacial Cleft Studies. Published 2022. https://www.dental.pitt.edu/research/ccdg/participate-research/pittsburgh-orofacial-cleft-studies
26. Faria ÁCO. *Fatores de Susceptibilidade Às Fissuras Orofaciais*. Universidade de São Paulo; 2019.
27. Costa VCR, Silva RC, Oliveira IF De, Paz B, Pogue R, Gazzoni L. Aspectos etiológicos e clínicos das fissuras labiopalatinas. *Revista de Medicina e Saúde de Brasília*. Published online 2018.
28. Silva AM da, Calumby RT, Freitas VS. Epidemiologic profile and prevalence of live births with orofacial cleft in Brazil: a descriptive study. *Revista Paulista de Pediatria*. 2023;42. doi:10.1590/1984-0462/2024/42/2022234
29. Wyszynski DF, Sarkozi A, Vargha P, Czeizel AE. Birth weight and gestational age of newborns with cleft lip with or without cleft palate and with isolated cleft palate. *J Clin Pediatr Dent*. 2003;27(2):185-190. doi:10.17796/jcpd.27.2.475367q2601u3x4w
30. Card EB, Morales CE, Kimia R, et al. A Retrospective and Prospective Cohort Study Comparing Pediatric Patients With Cleft Lip and Palate From the United States and Guatemala. *Journal of Craniofacial Surgery*. Published online July 14, 2023. doi:10.1097/SCS.0000000000009539

31. Nyarko KA, Lopez-Camelo J, Castilla EE, Wehby GL. Does the relationship between prenatal care and birth weight vary by oral clefts? Evidence using South American and united states samples. *Journal of Pediatrics*. 2013;162(1). doi:10.1016/j.jpeds.2012.06.040

32. Vandenbroucke JP, Elm von E, Altman D, et al. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. *Int J Surg 2014; 12: 1500-1524*. 2014;12:1500-1524.

33. Centers for Disease Control and Prevention. *User Guide to the 2021 Natality Public Use File*.; 2021.

34. Grünebaum A, McCullough LB, Orosz B, Chervenak FA. Neonatal mortality in the United States is related to location of birth (hospital versus home) rather than the type of birth attendant. *Am J Obstet Gynecol*. 2020;223(2):254.e1-254.e8. doi:10.1016/j.ajog.2020.01.045

35. Ministério da Saúde. *Declaração de Nascido Vivo Manual de Instruções Para Preenchimento*. http://bvsms.saude.gov.br/bvs/publicacoes/declaracao_nascido_vivo_manual_4ed.pdf

36. Oliveira MM de, Andrade SSC de A, Dimech GS, et al. Avaliação do Sistema de Informações sobre Nascidos Vivos. Brasil, 2006 a 2010. *Epidemiologia e Serviços de Saúde*. 2015;24(4):629-640. doi:10.5123/s1679-49742015000400005

37. Watkins SE, Meyer RE, Strauss RP, Aylsworth AS. Classification, Epidemiology, and Genetics of Orofacial Clefts. *Clin Plast Surg*. 2014;41(2):149-163. doi:10.1016/j.cps.2013.12.003

38. Romero DE, Cunha CB. Avaliação da qualidade das variáveis epidemiológicas e demográficas do Sistema de Informações sobre Nascidos Vivos , 2002. *Cad Saúde Públic*. 2007;23(3):701-714.

39. Nasreddine G, Hajj J El, Ghassibe-sabbagh M. Orofacial clefts embryology, classification, epidemiology, and genetics. *Mutat Res*. 2021;787(108373). doi:10.1016/j.mrrev.2021.108373

40. Salari N, Darvishi N, Heydari M, Bokaee S, Darvishi F, Mohammadi M. Global prevalence of cleft palate, cleft lip and cleft palate and lip: A comprehensive systematic review and meta-analysis. *J Stomatol Oral Maxillofac Surg*. 2022;123(2):110-120. doi:10.1016/j.jormas.2021.05.008

41. Mai CT, Isenburg JL, Canfield MA, et al. National population-based estimates for major birth defects, 2010–2014. *HHS Public Access*. 2019;111(18):1420-1435. doi:10.1002/bdr2.1589.National

42. Mossey PPA, Modell B. Epidemiology of Oral Clefts 2012: An International Perspective. *Epidemiology, Aetiology and Treatment Front Oral Biol*. 2012;16:1-18. doi:10.1159/000337464

43. Lidral AC, Moreno LM, Bullard SA. Genetic Factors and Orofacial Clefting. *Semin Orthod*. 2008;14(2):103-114. doi:10.1053/j.sodo.2008.02.002

44. Moura JR, Andrade APEDN, da Silva CAL, Freitas VS, Mercês ECD. Clinical-epidemiological profile of orofacial fissures in a reference center from northeast Brazil. *Revista de Salud Publica*. 2019;21(2):209–216.

45. de Souza Maurique L, Muniz FWMG, Silveira NPV, Camassola M, de Oliveira BM. Higher maternal age is associated with higher occurrence of cleft lip/palate in neonates under intensive care. *Braz J Oral Sci*. 2023;22. doi:10.20396/bjos.v22i00.8669246

46. Shehan JN, Danis DO, Bains A, Basa K, Marston AP, Levi JR. Cleft Palate in Newborns Diagnosed With Prematurity. *Otolaryngology - Head and Neck Surgery (United States)*. 2021;165(6):887-894. doi:10.1177/01945998211002148

47. Banik A, Kandilya D, Ramya S, Stünkel W, Chong YS, Thameem Dheen S. Maternal factors that induce epigenetic changes contribute to neurological disorders in offspring. *Genes (Basel)*. 2017;8(6):2-25. doi:10.3390/genes8060150

48. Viena CS, Sá J, Veiga PC, Coletta R, Medrado AP, Reis SR. Epidemiological Study of Cleft Palate in the State of Bahia, Brazil. *Brazilian Journal of Medicine and Human Health*. 2017;5(4):123-133. doi:10.17267/2317-3386bjmhh.v5i4.1337

49. Rodrigues L dos S, Lima RH da S, Costa LC, Batista RFL. Características das crianças nascidas com malformações congênitas no município de São Luís, Maranhão, 2002-2011. *Epidemiologia e Serviços de Saúde*. 2014;23(2):295-304. doi:10.5123/s1679-49742014000200011

50. Cavalcanti AL, Catão E de S, Fernandes TV, et al. An ecological study on orofacial clefts in northeastern Brazil. *Acta Facultatis Medicae Naissensis*. 2019;36(2):102-109. doi:10.5937/afmnai19021021

51. Figueirêdo CJR, Vasconcelos WKS, Maciel SSS V., Maciel WV, Gondim LAM, Tassitano RM. Prevalence of oral clefts in the State of Rio Grande do Norte , Brazil , between 2000-2005. *Rev Paul Pediatr*. 2011;29(1):29-34.

52. Pena SDJ, di Pietro G, Fuchshuber-Moraes M, et al. The genomic ancestry of individuals from different geographical regions of Brazil is more uniform than expected. *PLoS One*. 2011;6(2). doi:10.1371/journal.pone.0017063

53. Oliveira LQR de, Nicolau HC de S, Martelli DRB, et al. Ethnic Differences in the Brazilian Population Influence the Impact of BMP4 Genetic Variants on Susceptibility of Nonsyndromic Orofacial Clefts. *Cleft Palate Craniofacial Journal*. Published online 2023. doi:10.1177/10556656231180086

54. do Rego Borges A, Sá J, Hoshi R, et al. Genetic risk factors for nonsyndromic cleft lip with or without cleft palate in a Brazilian population with high African ancestry. *Am J Med Genet A*. 2015;167(10):2344-2349. doi:10.1002/ajmg.a.37181

55. Martelli DRB, Machado RA, Swerts MSO, Rodrigues LAM, de Aquino SN, Martelli-Júnior H. Non sindromic cleft lip and palate: Relationship between sex and clinical extension. *Braz J Otorhinolaryngol*. 2012;78(5):116-120. doi:10.5935/1808-8694.20120018

56. Impellizzeri A, Giannantoni I, Polimeni A, Barbato E, Galluccio G. Epidemiological characteristic of Orofacial clefts and its associated congenital anomalies: Retrospective study. *BMC Oral Health*. 2019;19(1):1-14. doi:10.1186/s12903-019-0980-5

57. Silva HPV da, Arruda TTS, Souza KSC de, et al. Risk factors and comorbidities in Brazilian patients with orofacial clefts. *Braz Oral Res*. 2018;32:e24. doi:10.1590/1807-3107bor-2018.vol32.0024

58. Bernheim N, Georges M, Malevez C, De Mey A, Mansbach A. Embryology and epidemiology of cleft lip and palate. *B-ENT*. 2006;2(Supp.l 4):11-19.

59. Sreejith VP, Arun V, Devarajan AP, Gopinath A, Sunil M. Psychological effect of prenatal diagnosis of cleft lip and palate: A systematic review. *Contemp Clin Dent*. 2018;9(2):304-308. doi:10.4103/ccd.ccd_673_17

60. Ministério da Saúde. *Atenção Ao Pré-Natal de Baixo Risco*. Vol 1. 32nd ed. (Ministério da Saúde, ed.).; 2013. www.saude.gov.br/dab

61. Rodrigues KMD, Zoldan C, Silva CB de O, Santana EFM, Júnior EA, Peixoto AB. Relationship between the number of prenatal care visits and the occurrence of adverse perinatal outcomes. *Rev Assoc Med Bras*. 2022;68(2):256-260. doi:10.1590/1806-9282.20211239

62. Wataganara T, Grunebaum A, Chervenak F, Wielgos M. Delivery modes in case of fetal malformations. *J Perinat Med*. 2017;45(3):273-279. doi:10.1515/jpm-2015-0364

63. Betrán AP, Ye J, Moller AB, Souza JP, Zhang J. Trends and projections of caesarean section rates: Global and regional estimates. *BMJ Glob Health*. 2021;6(6):1-8. doi:10.1136/bmjgh-2021-005671

64. Liu L, Oza S, Hogan D, et al. Global, regional, and national causes of under-5 mortality in 2000–15: an updated systematic analysis with implications for the Sustainable Development Goals. *The Lancet*. 2016;388(10063):3027-3035. doi:10.1016/S0140-6736(16)31593-8

65. Lei R lih, Chen H shys, Huang B yuan, et al. Population-Based Study of Birth Prevalence and Factors Associated with Cleft Lip and / or Palate in Taiwan 2002 – 2009. *PLoS One*. 2013;8(3):1-5. doi:10.1371/journal.pone.0058690

66. Phillips C, Velji Z, Hanly C, Metcalfe A. Risk of recurrent spontaneous preterm birth: A systematic review and meta-analysis. *BMJ Open*. 2017;7(6). doi:10.1136/bmjopen-2016-015402

67. Kruppa K, Krüger E, Vorster C, der Linde J van. Cleft Lip and/or Palate and Associated Risks in Lower-Middle-Income Countries: A Systematic Review. *Cleft Palate-Craniofacial Journal*. Published online 2021:1-9. doi:10.1177/10556656211018952

68. Sarilita E, Setiawan AS, Mossey PA. Orofacial clefts in low- and middle-income countries: A scoping review of quality and quantity of research based on literature between 2010-2019. *Orthod Craniofac Res*. 2021;24(3):421-429. doi:10.1111/ocr.12458

69. Medronho R de A, Bloch KV, Luiz RR, Werneck GL. *Epidemiologia*. 2nd ed. Atheneu; 2009.

70. Romero DE, Cunha CB. Disponibilidade e qualidade da informação epidemiológica e demográfica no Sistema de Informações sobre Nascidos Vivos (SINASC) e no Sistema de Informação de Mortalidade (SIM) para menores de um. *Anais ABEP*. 2006;(Xv):1-18.

71. Marengo L, Farag NH, Canfield M. Body mass index and birth defects: Texas, 2005-2008. *Matern Child Health J*. 2013;17(10):1898-1907. doi:10.1007/s10995-012-1214-5

72. Carmichael SL, Ma C, Tinker SC, Rasmussen SA, Shaw GM, National Birth Defects Prevention Study. Maternal stressors and social support as risks for delivering babies with structural birth defects. *HHS Public Access*. 2014;28(4):338-344. doi:10.1111/ppe.12123.Maternal

73. Block SR, Watkins SM, Salemi JL, et al. Maternal pre-pregnancy body mass index and risk of selected birth defects: Evidence of a dose-response relationship. *Paediatr Perinat Epidemiol*. 2013;27(6):521-531. doi:10.1111/ppe.12084

74. Wu Y, Liu B, Sun Y, et al. Association of maternal prepregnancy diabetes and gestational diabetes mellitus with congenital anomalies of the newborn. *Diabetes Care*. 2020;43(12):2983-2990. doi:10.2337/dc20-0261

75. Yang L, Wang H, Yang L, et al. Maternal cigarette smoking before or during pregnancy increases the risk of birth congenital anomalies: a population-based retrospective cohort study of 12 million mother-infant pairs. *BMC Med*. 2022;20(1). doi:10.1186/s12916-021-02196-x

76. Marinho F, Araújo VEM, Porto DL, et al. Microcephaly in Brazil: prevalence and characterization of cases from the Information System on Live Births (Sinasc), 2000-2015. *Epidemiol Serv Saúde*. 2016;25(4):701-712. doi:10.5123/S1679-49742016000400004

77. Rodrigues K, Sena MF de, Roncalli ÂG, Ferreira MÂF. Prevalence of orofacial clefts and social factors in Brazil. *Braz Oral Res*. 2009;23(1):38-42. doi:10.1590/s1806-83242009000100007

78. Almeida NKO, Almeida RMVR, Pedreira CE. Adverse perinatal outcomes for advanced maternal age: A cross-sectional study of Brazilian births. *J Pediatr (Rio J)*. 2015;91(5):493-498. doi:10.1016/j.jped.2014.12.002

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ORIGINAL ARTICLE

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Polymorphisms associated with oral clefts as potential markers for oral pre and malignant disorders

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Abstract

Objective: To investigate whether genes in the Wnt pathway, which have been previously associated with both oral clefts and oral squamous cell carcinoma, are also associated with oral potentially malignant disorders (leukoplakia, erythroplakia and lichen planus).

Materials and Methods: Case–control study: Dataset consisted of clinical information linked to DNA samples from affected subjects diagnosed with oral potential malignant disorders and oral cancer and their matched controls. Individual samples, clinical history, and potential risk factors were obtained through the Dental Registry and DNA Repository project of the School of Dental Medicine, University of Pittsburgh. The rs1533767 (WNT11), rs9879992 (GSK3B), and rs3923087 (AXIN2) were tested. After genomic DNA had been extracted, genotyping was performed blindly to clinical diagnosis status. Representation of genotypes and alleles in affected subjects in comparison to the unaffected individuals was determined using PLINK. Additional analysis was performed to investigate associations between environmental (socioeconomic/lifestyle) risk factors and the oral pathologies studied using STATA.

Results: Two of the SNPs tested (rs9879992 in GSK3B and rs3923087 in AXIN2) were statistically, significantly associated with the pathologies studied ($p = 0.039$ and 0.038, respectively).

Conclusion: Single-nucleotide polymorphisms in genes in the Wnt pathway were associated with oral potentially malignant disorders.

KEYWORDS
cleft lip and palate, oral cancer, potentially malignant disorders of the mucosa

1 | INTRODUCTION

The Wnt (Wingless-related integration site) signaling pathway is an evolutionarily conserved signal pathway that regulates several biological processes. These include cell migration, cell polarity, neural and organ formation during embryonic development, and cell regeneration and maintenance during life (Ejaz & Ghafoor, 2019). Without Wnt proteins, the intracellular β-catenin is degraded in the cytoplasm by a β-catenin destruction complex, which includes proteins such as *AXIN* and *GSK3*. However, when Wnt proteins are present, they bind to the extracellular frizzled receptor and trigger a series of events that target the destruction of the β-catenin complex, allowing an increase in the amount of intracellular β-catenin. The β-catenin is translocated to the nucleus, leading to the transcription of Wnt genes, which are responsible for cell growth and proliferation (Komiya & Habas, 2008). Disruptions in the Wnt signaling pathway are related to some skeletal defects, fibrotic diseases, inflammatory disorders and malignant transformations of multiple cancers (Ejaz & Ghafoor, 2019).

Oral Squamous Cell Carcinoma (OSCC) is the most common oral malignancy worldwide and is a major public health concern, and its early diagnosis is essential to improve patient survival and reduce

mortality rates (Chen et al., 2021). It is difficult to detect cancer in its early stages, so focus has been placed on the identification of cancer risk markers, which can be genetic variants or phenotypic characteristics that indicate a genetic predisposition in the development of the disease (Bezamat et al., 2020). It has been suggested that single-nucleotide polymorphisms (SNPs) at genes of the Wnt pathway, such as *AXIN2*, *WNT11*, *GSK3B*, predispose to orofacial clefts (OC) during embryological development and to oral cancer in any stage of life (Andrade Filho et al., 2011; de Freitas et al., 2019; Letra et al., 2009; Menezes et al., 2009; Purwaningsih et al., 2021). Also, phenotypic risk markers, such as oral potentially malignant disorders (OPMD) (Ranganathan & Kavitha, 2019; Shearston et al., 2019) and OC (Bille et al., 2010; Vieira, 2020, 2021) have been found to be associated with OSCC.

Oral potentially malignant disorders are defined as any oral mucosal abnormality that is associated with a statistically increased risk of developing oral cancer and include leukoplakia, erythroplakia, oral lichen planus, oral submucous fibrosis, palatal lesions in reverse smokers, lupus erythematosus, epidermolysis bullosa and dyskeratosis congenita (Warnakulasuriya et al., 2021). A study designed to identify orofacial conditions that can serve as potential risk markers for cancer showed that when analyzing the individuals affected by cancer, leukoplakia of oral mucosa was associated with *AXIN2*, suggesting that this phenotype predisposes to higher cancer risk (Bezamat et al., 2020). Also, there must be a role for β-catenin protein in the progression of dysplasia and early malignant transformation to OSCC (Reyes et al., 2015).

These findings lead to the hypotheses that the identification of different phenotypes (OPMD) associated with specific polymorphisms that may also be associated with oral cancer and OC could help to determine which patients are at higher risk for developing these conditions. Based on that, this study aimed to investigate whether genes in the Wnt pathway, which have been previously associated with both orofacial clefts and oral squamous cell carcinoma, are also associated with oral potentially malignant disorders (leukoplakia, erythroplakia and lichen planus).

2 | MATERIALS AND METHODS

This is a cohort study developed with a dataset consisting of clinical information linked to DNA samples from 822 subjects, who sought treatment at the University of Pittsburgh dental clinics. These subjects were screened from a total of 6982 subjects. The cases consisted of 274 affected subjects diagnosed with OPMD (248 with Leukoplakia, 4 with Erytroleukoplakia or 10 with Lichen Planus) and oral cancer (12 subjects). They were matched by sex and age in a 1:2 ratio with a comparison group of unrelated subjects without a history of any oral malignant disorder or cancer (Figure S1). This study has the approval of the University of Pittsburgh Institutional Review Board (IRB # 19050020).

Individual samples, clinical history, and potential risk factors were obtained through the Dental Registry and DNA Repository project (DRDR Project) of the School of Dental Medicine, University of Pittsburgh. This database is composed by the registry of clinical information, including a complete list of oral conditions and treatments, and biological sample collection from subjects who seek care at the dental clinics of the University of Pittsburgh. All clinical data are recorded following the same guidelines by dental students in training under the supervision of experienced dental professionals who are calibrated annually. The clinical data extracted for this study included phenotypes (OPMD – leukoplakia, erythroplakia, oral lichen planus – and oral cancer), and environmental (socioeconomic – educational level and ethnicity – and lifestyle – smoking and alcohol consumption) risk factors.

Three SNPs from three Wnt pathway genes that have been previously suggested to be associated with orofacial clefts (OC) and oral cancer (Andrade Filho et al., 2011; de Freitas et al., 2019). They were rs1533767 (*WNT11*), rs9879992 (*GSK3B*), and rs3923087 (*AXIN2*). Information on the SNPs (variant type, alleles, functional consequence and minor allele frequency) was obtained from the NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/snp). The ALFA project provides global aggregate allele frequency from dbGaP (Table S1).

After the genomic DNA had been extracted from saliva samples using established protocols, genotyping was performed blindly to clinical diagnosis status. Reactions were carried out using TaqMan chemistry (Ranade et al., 2001) in the GeneAmp PCR System 9700 (Applied Biosystems). Applied Biosystems supplied assays and reagents. Endpoint analysis of PCR products was performed using the QuantStudio 6 Flex (Applied Biosystems). PCR reactions were repeated twice when necessary.

Hardy–Weinberg equilibrium (HWE) was assessed through the chi-square test, assuming *p*-values above 0.01 (0.05/3; the denominator is the number of genetic markers) as statistically significant. Over representation of genotypes and alleles in affected subjects in comparison to the unaffected matched individuals were determined. Considering D as the minor allele and d is the major allele, the genetic models included were the allelic (D×d), the genotypic model (DD×Dd×dd), the dominant model (dd×DD+Dd), and the recessive model (DD×Dd+dd) (Bezamat et al., 2022). Logistic regression models were performed to obtain the adjusted Odds Ratio (OR) regarding the alleles and outcomes associations, adjusting for smoking habits and alcohol. Genetic analyzes were done at Plink Software, assuming a *p*-value below 0.05 and a 95% Confidence Interval (95% CI) as statistically significant. Also, in order to investigate possible associations between environmental risk factors and the oral pathologies studied, an additional analysis was performed using the STATA software, assuming a *p*-value below 0.05 and a 95% CI as statistically significant.

3 | RESULTS

We found a statistically significant association between two of the SNPs tested (rs9879992 in *GSK3B* and rs3923087 in *AXIN2*)

and the pathologies studied (Table S2). Both SNPs showed a genotypic association (*p*-values 0.039 and 0.038, respectively). Also, rs9879992 in *GSK3B* was statistically associated through recessive model (OR = 1.88, *p* = 0.024, 95% CI = 1.081–3.284) while rs3923087 in *AXIN2* was statistically associated through allelic (OR = 1.36, *p* = 0.003, 95% CI = 1.105–1.69), dominant (OR = 0.72, *p* = 0.031, 95% CI = 0.536–0.972), and recessive (OR = 1.48, *p* = 0.025, 95% CI = 0.049–2.11) models. The third SNPs tested (rs153767 in *WNT11*) did not show statistically significant difference in the frequency with the phenotypes studied by any of the genetic analyses realized. The logistic regression models showed that the rs3923087/T was still associated with an increased risk for OPMD/oral cancer, even after adjust by the covariates smoking and alcohol (adjusted OR = 1.29, *p* = 0.008; adjusted OR = 1.28, 0.010, respectively) (Table S3).

The genotype and allele frequencies found were consistent with those expected by the HWE. Also, the allele frequencies found were in agreement with the aggregate allele frequency from dbGaP. The only exception was a distortion in HWE for the rs3923087 in AXIN2 in the control group, suggesting loss-of-heterozygosity (LOH).

We compared the frequency of potential environmental (socioeconomic – educational level and ethnicity – and lifestyle – smoking and alcohol consumption) risk factors for both OC and oral cancer between subjects diagnosed with and without OPMD/oral cancer. Only smoking habits remained statistically associated with OPMD/oral cancer after adjustment for the other variables (adjusted OR = 2.22, *p* = 0.000 95% CI = 1.535–3.217; Table S4).

4 | DISCUSSION

This study investigated if SNPs in the Wnt genes pathway, which have been previously associated with both OC and OSCC (Andrade Filho et al., 2011; de Freitas et al., 2019), are also associated with OPMD development. Our results showed an association between genetic markers rs9879992 in *GSK3B* and rs3923087 in *AXIN2* and OPMD/oral cancer (*p*-values 0.039 and 0.038, respectively). These results can assist identifying OPMD patients with an increased risk of malignant transformation, as supported by the literature (Shearston et al., 2019). In addition, since these SNPs were associated with OC, OSCC (Andrade Filho et al., 2011; de Freitas et al., 2019), and also to OPMD, it could be suggested that variations in Wnt genes that were shown to be involved with OC may also increase the susceptibility of developing OPMD or OSCC. They are three different phenotypes that share similar variations in Wnt genes, which are involved in cell proliferation and differentiation (Andrade Filho et al., 2011; Chiquet et al., 2008; de Freitas et al., 2019; Ejaz & Ghafoor, 2019; Menon, 2017; Zhan et al., 2017).

The location and function of AXIN and GSK3B have important effects on the dynamics of Wnt signaling (Liu & Millar, 2010). Our results showed that for both SNPs (rs9879992 in *GSK3B* and rs3923087 in *AXIN2*) the rare homozygous genotype (GG and TT, respectively) was more frequent in individuals diagnosed with OPMD/oral cancer as compared with controls. There was a significant association of an intronic SNP in GSK3B (rs9879992) with OPMD/oral cancer. Here, this SNPs to be associated also through the recessive model, meaning that the frequency of two copies of the rare allele G was associated with a risk of 1.88 (*p* = 0.024, 95% CI = 1.081–3.284) of having OPMD/oral cancer versus the sum of the other genotypes. This association remained statistically significant after adjustments for smoking and alcohol consumption (adjusted OR = 1.29, *p* = 0.008; adjusted OR = 1.28, 0.010, respectively). Previously studies found similar results, showing the association of the minor allele G and oral cancer/ oral squamous cell carcinoma (OR: 1.67, 95% CI: 1.07–2.59, *p* = 0.02 [de Freitas et al., 2019] and OR = 1.78, 95% CI: 1.27–2.5, *p* = 0.0007 [Andrade Filho et al., 2011]).

Regarding the rs3923087 (in *AXIN2*), the presence of at least one copy of the minor allele T was associated with a risk of 1.36 (*p* = 0.003, 95% CI = 1.105–1.69) of having OPMD/oral cancer. The dominant and recessive models imply that the presence of two copies of the major allele C had a protective effect (OR = 0.72, *p* = 0.031, 95% CI = 0.536–0.972) while the presence of two copies of the minor allele T increased the risk (OR = 1.48, *p* = 0.025, 95% CI = 0.049–2.11) of having OPMD/oral cancer. The association we present here has been reported earlier (Andrade Filho et al., 2011) also for a population from Pittsburgh, but showing a protective effect for the minor allele T. This can be explained by either some differences in the populational substructure of the two samples, which may have had differences in distributions of Northern European descents or African Americans, or by the presence of linkage disequilibrium, and the contributing variant is not the rs3923097 studied but another variant or variants nearby. Additionally, an increased abundance of AXIN2 was highly correlated to the malignant transformation of oral leukoplakia (Zhang et al., 2017). The AXIN2 when mutated is shown to increase the susceptibility to colon cancer, and it is also associated with orofacial clefts (Menezes et al., 2009).

This is the first study that provided evidence for association between gene OPMD and genes of Wnt also related to OSCC and OC occurrence. Commonly studied SNPs for OPMD were those related to genes of carcinogen metabolism, DNA repair, cell cycle control, extra-cellular matrix alteration and immune-inflammatory pathways (Shridhar et al., 2016). When it was focused in data regarding Wnt pathway genes and OPMD the literature is scarce. There is a phenome-wide scan study in that oral leukoplakia was associated with *AXIN2*, predisposing a higher cancer risk (Bezamat et al., 2020), and some immunohistochemical studies that suggested a role for β-catenin in the progression of oral dysplasia (Reyes et al., 2015, 2019) and oral leukoplakia (Ishida et al., 2007).

It was found a distortion in the HWE for the rs3923087 in *AXIN2*, which can be a consequence of LOH. This condition occurs when a somatic mutation leads to the loss of a portion of one of the parental homologs (Wilkins & Laframboise, 2011), such as a microdeletion in one of the chromosomes. Microdeletion analysis showed the involvement of LOH in several cancers, such as breast, lung, neuroblastoma, OSCC, cervical, thyroid, prostate, leukemia and head and

neck tissues (Cengiz et al., 2005). The LOH of certain chromosomal loci predisposes the transformation of OPMDs in malignant lesion (Farah et al., 2019; Lingen et al., 2011; Lorini et al., 2021; Zhang & Sjöblom, 2021) since it interferes in the Wnt pathway mechanisms of cell growth and differentiation (Purwaningsih et al., 2021). The increased cytoplasmic expression of *AXIN2* in oral leukoplakia lesions is associated with the malignancy transformation of these lesions (Ejaz & Ghafoor, 2019). Also, the *AXIN2* gene shows frequent LOH in some types of cancer (https://www.ncbi.nlm.nih.gov/gene/?term=rs3923087). It was found in the literature deviations in HWE regarding variations in Wnt family genes when analyzed their relationship with cancer (Bahl et al., 2017; de Freitas et al., 2019). Also, we have seen deviations in HWE in previous studies exploring associations between genetic variants and oral clefts depending on other covariates such as smoking or folic acid intake (Assis et al., 2020; Prescott & Malcolm, 2002).

In addition to genetic predisposition to developed OC and OPMD/cancer, it should be also considered environmental factors that may modulate gene expression, playing a role in the risk to develop the disease, such as smoking and alcohol intake, and others. In our study, when socioeconomic and environmental risks were analyzed, only smoking was statistically associated with OPMD/OSCC, showing a risk twice higher among the case group (adjusted OR = 2.22, $p = 0.000$, 95% CI = 1.535–3.217). This association was expected, since the contribution of smoking in increase the risk to develop precancerous oral lesions and oral cancer is frequent reported (Kindler et al., 2021; Wetzel & Wollenberg, 2020). Also, maternal smoking during pregnancy shows to be an important risk factor in the OC etiology of CL/P (Inchingolo et al., 2022; Little et al., 2004). Although the lack of association between alcohol and OPMD/oral cancer in our study, alcohol consumption is consistently linked to an increase risk of oral premalignant lesions (Wetzel & Wollenberg, 2020), OSCC (Rahman et al., 2020). For OC, the correlation between alcohol consumption during pregnancy and its occurrence has some divergency, but precautions should be taken to avoid it during the first trimester (Yin et al., 2019).

5 | CONCLUSION

In conclusion, single nucleotides polymorphisms in Wnt genes – rs9879992 in *GSK3B* and rs3923087 in *AXIN2* – were statistically significant associated with oral potentially malignant disorders/oral squamous cell carcinoma. These results reinforce the suggested implications for this gene family in malignant transformation of oral disorders and in cancer development. Also, considering the contribution of these genes to orofacial cleft development, the results of this study corroborate with suggestions that the same genes may have different roles during different biological processes, predisposing to orofacial cleft, when alterations occur in these genes during embryonic development, and to cancer, when they occur later in life, modulated by environmental exposures such as smoking habits.

AUTHOR CONTRIBUTIONS

Adriana Mendonça da Silva: Conceptualization; investigation; writing – original draft; methodology; writing – review and editing; data curation; visualization; formal analysis. **Valéria Souza Freitas:** Supervision; conceptualization; methodology. **Alexandre Rezende Vieira:** Conceptualization; methodology; writing – review and editing; supervision; resources; project administration; funding acquisition; visualization; formal analysis.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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REFERENCES

Andrade Filho, P. A., Letra, A., Cramer, A., Prasad, J. L., Garlet, G. P., Vieira, A. R., Ferris, R. L., & Menezes, R. (2011). Insights from studies with oral cleft genes suggest associations between WNT-pathway genes and risk of oral cancer. *Journal of Dental Research*, *90*(6), 740–746. https://doi.org/10.1177/0022034511401622

Assis, I. O., Lacerda, R. H. W., Cavalcante, B. G. N., Bezamat, M., Modesto, A., & Vieira, A. R. (2020). IRE1 less common homozygous genotype in families with positive history of cancer and individuals born with cleft lip/palate. *The Journal of Craniofacial Surgery*, *32*(5), e407–e411. https://doi.org/10.1097/SCS.0000000000007169

Bahl, C., Sharma, S., Singh, N., & Behera, D. (2017). Association study between genetic variations in Axin2 gene and lung cancer risk in North Indian population: A multiple interaction analysis. *Tumor Biology*, *39*(4), 1–18. https://doi.org/10.1177/1010428317695533

Bezamat, M., Harrison, B., Zhou, Y., Glickman, K. M., Telles, V., Guirguis, C., Modesto, A., & Vieira, A. R. (2020). Phenome-wide scan finds potential orofacial risk markers for cancer. *Scientific Reports*, *10*(1), 1–10. https://doi.org/10.1038/s41598-020-61654-3

Bezamat, M., Rothenberger, S., & Vieira, A. R. (2022). Genetic contribution to cancer risk in patients with tooth loss: A genetic association study. *Scientific Reports*, *12*(1), 1–7. https://doi.org/10.1038/s41598-022-20556-2

Bille, C., Winther, J. F., Bautz, A., Murray, J. C., Olsen, J., & Christensen, K. (2010). Cancer risk in persons with oral cleft—A population-based study of 8,093 cases. *NIH Public Access*, *161*(11), 1047–1055. https://doi.org/10.1093/aje/kwi132

Cengiz, B., Gunduz, M., Gunduz, E., Shimizu, K., Nagai, N., Ouchida, M., & Inoue, T. (2005). Deletional mapping of 2q21-37 region in oral cancer. *Journal of Hard Tissue Biology*, *14*(2), 227–228. https://doi.org/10.2485/jhtb.14.227

Chen, S. H., Hsiao, S. Y., Chang, K. Y., & Chang, J. Y. (2021). New insights into oral squamous cell carcinoma: From clinical aspects to molecular tumorigenesis. *International Journal of Molecular Sciences*, *22*(5), 1–21. https://doi.org/10.3390/ijms22052252

Chiquet, B. T., Blanton, S. H., Burt, A., Ma, D., Stal, S., Mulliken, J. B., & Hecht, J. T. (2008). Variation in WNT genes is associated with

non-syndromic cleft lip with or without cleft palate. *Human Molecular Genetics*, *17*(14), 2212–2218. https://doi.org/10.1093/hmg/ddn121

de Freitas, E. M., Machado, R. A., de Moura Santos, E., de Matos, F. R., Galvão, H. C., Miranda Soares, P. B., Freitas, R. A., & Martelli-Júnior, H. (2019). Polymorphisms associated with oral clefts as potential susceptibility markers for oral and breast cancer. *Archives of Oral Biology*, *99*, 9–14. https://doi.org/10.1016/j.archoralbio.2018.12.004

Ejaz, I., & Ghafoor, S. (2019). Wnt signaling pathway in oral lesions. *Journal of the Pakistan Medical Association*, *69*(11), 1687–1692. https://doi.org/10.5455/jpma.5890

Farah, C. S., Shearston, K., Nguyen, A. P., & Kujan, O. (2019). Oral carcinogenesis and malignant transformation. In P. Brennan, T. Aldridge, & R. Dwivedi (Eds.), *Premalignant conditions of the oral cavity* (pp. 27–66). Springer. https://doi.org/10.1007/978-981-13-2931-9_3

Inchingolo, A. M., Fatone, M. C., Malcangi, G., Avantario, P., Piras, F., Patano, A., Di Pede, C., Netti, A., Ciocia, A. M., De Ruvo, E., Viapiano, F., Palmieri, G., Campanelli, M., Mancini, A., Settanni, V., Carpentiere, V., Marinelli, G., Latini, G., Rapone, B., … Dipalma, G. (2022). Modifiable risk factors of non-syndromic orofacial clefts: A systematic review. *Children*, *9*(12), 1846.

Ishida, K., Ito, S., Wada, N., Deguchi, H., Hata, T., Hosoda, M., & Nohno, T. (2007). Nuclear localization of beta-catenin involved in precancerous change in oral leukoplakia. *Molecular Cancer*, *6*, 1–7. https://doi.org/10.1186/1476-4598-6-62

Kindler, S., Samietz, S., Dickel, S., Mksoud, M., Kocher, T., Lucas, C., Seebauer, C., Doberschütz, P., Holtfreter, B., Völzke, H., Metelmann, H. R., & Ittermann, T. (2021). Prevalence and risk factors of potentially malignant disorders of the mucosa in the general population: Mucosa lesions a general health problem? *Annals of Anatomy*, *237*, 151724. https://doi.org/10.1016/j.aanat.2021.151724

Komiya, Y., & Habas, R. (2008). Wnt signal transduction pathways. *Organogenesis*, *4*(2), 68–75.

Letra, A., Menezes, R., Granjeiro, J. M., & Vieira, A. R. (2009). AXIN2 and CDH1 polymorphisms, tooth agenesis, and oral clefts. *Birth Defects Research Part A – Clinical and Molecular Teratology*, *85*(2), 169–173. https://doi.org/10.1002/bdra.20489

Lingen, M., Pinto, A., Mendes, R., Franchini, R., Czerninski, R., Tilakaratne, W., Partridge, M., Peterson, D. E., & Woo, S. B. (2011). Genetics/epigenetics of oral premalignancy: Current status and future research. *Oral Diseases*, *17*(Suppl. 1), 7–22. https://doi.org/10.1111/j.1601-0825.2011.01789.x

Little, J., Cardy, A., & Munger, R. G. (2004). Tobacco smoking and oral clefts: A meta-analysis. *Bulletin of the World Health Organization*, *82*(3), 213–218. https://doi.org/10.1590/S0042-96862004000300011

Liu, F., & Millar, S. E. (2010). Wnt/β-catenin signaling in oral tissue development and disease. *Journal of Dental Research*, *89*(4), 318–330. https://doi.org/10.1177/0022034510363373

Lorini, L., Atín, C. B., Thavaraj, S., Müller-Richter, U., Ferranti, M. A., Romero, J. P., Sáez Barba, M., de Pablo García-Cuenca, A., Braña García, I., Bossi, P., Nuciforo, P., & Simonetti, S. (2021). Overview of oral potentially malignant disorders: From risk factors to specific therapies. *Cancers*, *13*(15), 1–16. https://doi.org/10.3390/cancers13153696

Menezes, R., Marazita, M. L., McHenry, T. G., Cooper, M. E., Bardi, K., Brandon, C., Letra, A., Martin, R. A., & Vieira, A. R. (2009). AXIS inhibition protein 2, orofacial clefts and a family history of cancer. *Journal of the American Dental Association*, *140*(1), 80–84. https://doi.org/10.14219/jada.archive.2009.0022

Menon, R. S. (2017). *Wnt signaling in oral cancer initiating cells* (Doctoral Dissertation). Harvard School of Dental Medicine. https://doi.org/10.1016/j.oooo.2017.06.015

Purwaningsih, N. M. S., Khor, G. H., Nik Mohd Rosdy, N. M. M., & Abdul Rahman, E. O. (2021). Wnt pathway in oral cancer: A review update. *Saudi Dental Journal*, *33*(8), 813–818. https://doi.org/10.1016/j.sdentj.2021.08.002

Prescott, N. J., & Malcolm, S. (2002). Folate and the face: Evaluating the evidence for the influence of folate genes on craniofacial development. *The Cleft Palate-Craniofacial Journal*, *39*(3), 327–331. https://doi.org/10.1597/1545-1569_2002_039_0327_fatfet_2.0.co_2

Rahman, Q. B., Iocca, O., Kufta, K., & Shanti, R. M. (2020). Global burden of head and neck cancer. *Oral and Maxillofacial Surgery Clinics of North America*, *32*(3), 367–375. https://doi.org/10.1016/j.coms.2020.04.002

Ranade, K., Chang, M. S., Ting, C. T., Pei, D., Hsiao, C. F., Olivier, M., Pesich, R., Hebert, J., Chen, Y. D., Dzau, V. J., Curb, D., Olshen, R., Risch, N., Cox, D. R., & Botstein, D. (2001). High-throughput genotyping with single nucleotide polymorphisms. *Genome Research*, *11*(7), 1262–1268. https://doi.org/10.1101/gr.157801

Ranganathan, K., & Kavitha, L. (2019). Oral epithelial dysplasia: Classifications and clinical relevance in risk assessment of oral potentially malignant disorders. *Journal of Oral and Maxillofacial Pathology*, *23*(1), 19–27. https://doi.org/10.4103/jomfp.JOMFP

Reyes, M., Peña-Oyarzuna, D., Maturanac, A., & Torres, V. A. (2019). Nuclear localization of β-catenin and expression of target genes are associated with increased Wnt secretion in oral dysplasia. *Oral Oncology*, *94*, 58–67.

Reyes, M., Rojas-Alcayaga, G., Maturana, A., Aitken, J. P., Rojas, C., & Ortega, A. V. (2015). Increased nuclear β-catenin expression in oral potentially malignant lesions: A marker of epithelial dysplasia. *Medicina Oral, Patología Oral y Cirugía Bucal*, *20*(5), e540–e546. https://doi.org/10.4317/medoral.20341

Shearston, K., Fateh, B., Tai, S., Hove, D., & Farah, C. S. (2019). Malignant transformation rate of oral leukoplakia in an Australian population. *Journal of Oral Pathology and Medicine*, *48*(7), 530–537. https://doi.org/10.1111/jop.12899

Shridhar, K., Aggarwal, A., Walia, G. K., Gulati, S., Geetha, A. V., Prabhakaran, D., Dhillon, P. K., & Rajaraman, P. (2016). Single nucleotide polymorphisms as markers of genetic susceptibility for oral potentially malignant disorders risk: Review of evidence to date. *Oral Oncology*, *61*, 146–151. https://doi.org/10.1016/j.oraloncology.2016.08.005

Vieira, A. R. (2020). Genetic basis of oral health conditions. In *Genetic Basis of Oral Health Conditions*. Springer. https://doi.org/10.1007/978-3-030-14485-2

Vieira, A. R. (2021). Cleft lip/palate and cancer: A true connection. *Revista Brasileira de Cancerologia*, *67*(4), 11–13.

Warnakulasuriya, S., Kujan, O., Aguirre-Urizar, J. M., Bagan, J. V., González-Moles, M. Á., Kerr, A. R., Lodi, G., Mello, F. W., Monteiro, L., Ogden, G. R., Sloan, P., & Johnson, N. W. (2021). Oral potentially malignant disorders: A consensus report from an international seminar on nomenclature and classification, convened by the WHO Collaborating Centre for Oral Cancer. *Oral Diseases*, *27*(8), 1862–1880. https://doi.org/10.1111/odi.13704

Wetzel, S. L., & Wollenberg, J. (2020). Oral potentially malignant disorders. *Dental Clinics of North America*, *64*(1), 25–37. https://doi.org/10.1016/j.cden.2019.08.004

Wilkins, K., & Laframboise, T. (2011). Losing balance: Hardy-Weinberg disequilibrium as a marker for recurrent loss-of-heterozygosity in cancer. *Human Molecular Genetics*, *20*(24), 4831–4839. https://doi.org/10.1093/hmg/ddr422

Yin, X., Li, J., Li, Y., & Zou, S. (2019). Maternal alcohol consumption and oral clefts: A meta-analysis. *British Journal of Oral and Maxillofacial Surgery*, *57*(9), 839–846. https://doi.org/10.1016/j.bjoms.2019.08.013

Zhan, T., Rindtorff, N., & Boutros, M. (2017). Wnt signaling in cancer. *Oncogene*, *36*(11), 1461–1473. https://doi.org/10.1038/onc.2016.304

Zhang, X., & Sjöblom, T. (2021). Targeting loss of heterozygosity: A novel paradigm for cancer therapy. *Pharmaceuticals*, *14*(1), 1–17. https://doi.org/10.3390/ph14010057

Zhang, X., Kim, K. Y., Zheng, Z., Kim, H. S., Cha, I. H., & Yook, J. I. (2017). Snail and Axin2 expression predict the malignant transformation of oral leukoplakia. *Oral Oncology*, *73*, 48–55. https://doi.org/10.1016/j.oraloncology.2017.08.004

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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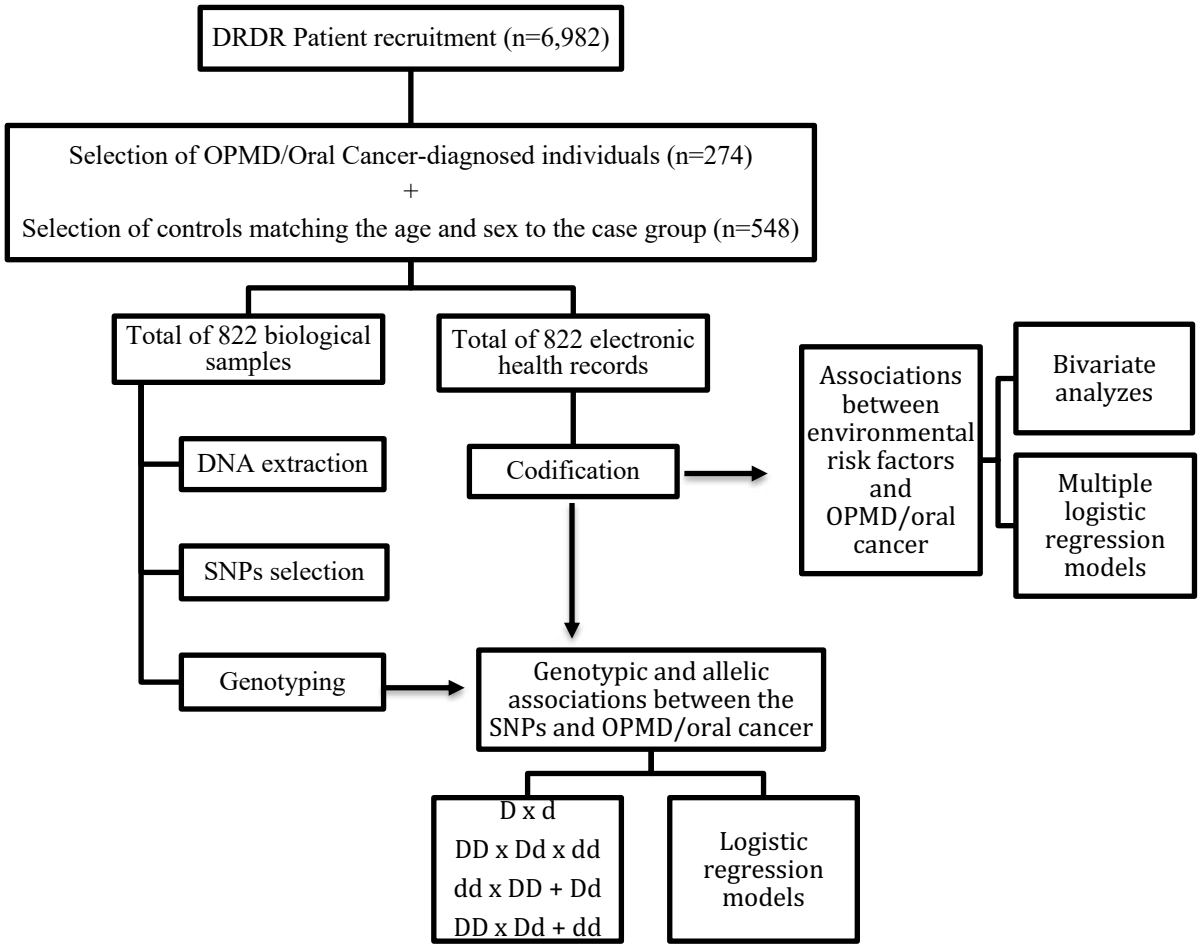
TABLES AND FIGURES (as supplementary material)

Figure 1 – Overall study design



OPMD – oral potentially malignant disorders
DRDR - Dental Registry and DNA Repository project
Environmental risk factors: socioeconomic (education and ethnicity) and lifestyle (smoking and alcohol consumption)

Table 1 - Summary of Candidate Genes and SNPs Studied

Gene	Chromosome	SNP	SNP loci	Base change	Minor Allele (MAF)
WNT11	11	rs1533767	Exon	G>A	A (0.24)
GSK3B	3	rs9879992	Intron	A>G	G (0.25)
AXIN2	17	rs3923087	Intron	T>C	T (0.27)

MAF = Minor Allele Frequency (from dbGaP)

Table 2 - Results from the genetic analyses from OPMD/OSSC

Gene	SNP	Allele D/d	Model	Affected	Unaffected	X^2	DF	p value	OR [CI95%]
WNT11	rs1533767	A/G	Genotypic	19/94/157	35/198/309	0.279	2	0.870	-
			Allele	132/408	267/815	0.01	1	1	0.98 [0.777-1.255]
			Dominant	157/113	309/232	0.078	1	0.779	1.04 [0.776-1.402]
			Recessive	19/251	35/506	0.093	1	0.76	1.09 [0.614-1.952]
GSK3B	rs9879992	G/A	Genotypic	26/93/148	28/211/278	6.488	2	**0.039**	-
			Allele	145/389	267/767	0.322	1	0.570	1.07 [0.846-1.356]
			Dominant	119/148	239/278	0.195	1	0.658	1.06 [0.795-1.439]
			Recessive	26/241	28/489	5.128	1	**0.024**	**1.88[1.081-3.284]**
AXIN2	rs3923087	T/C	Genotypic	68/92/106	101/180/258	6.559	2	**0.038**	-
			Allele	228/304	382/696	8.336	1	**0.003**	**1.36[1.105-1.69]**
			Dominant	160/106	281/258	4.621	1	**0.031**	**0.72[0.536-0.972]**
			Recessive	68/198	101/438	5.003	1	**0.025**	**1.48[1.049-2.11]**

Bold indicates statistically significant p-values under the threshold 0.05
X^2 = Chi-square value
OR = Odds Ratio
DF = Degree of freedom
Allelic model (D x d), genotypic model (DD x Dd x dd), dominant model (dd x DD + Dd), and recessive model (DD x Dd + dd), assuming d as most common allele and D as the less common allele.

Table 3 - Logistic regression analysis of associations between the SNPs and OPMD/oral cancer

SNP/Minor allele	Adjustment	OR (adjusted)	p value
rs9879992/G	Smoking habits	1.066	0.599
	Alcohol consumption	1.071	0.569
rs1533767/A	Smoking habits	0.9801	0.868
	Alcohol consumption	0.9858	0.905
rs3923087/T	Smoking habits	1.291	**0.008**
	Alcohol consumption	1.281	**0.010**

Bold indicates statistically significant p-values under the threshold 0.05
OR = Odds Ratio

Table 4 - Unadjusted and adjusted OR of the associations between environmental risk factors and OPMD

Exposure	OR	p(95%C.I.)	OR (adjusted)	P value (95%C.I.)
Alcohol consumption (drinkers)	0.91	0.644(0.592-1.387)	0.76	0.266(0.482-1.223)
Smoking habits (smokers)	2.36	**0.000**(1.664-3.369)	2.22	**0.000**(1.535-3.217)
Ethnicity (White and Asian)	0.57	**0.002**(0.394-0.831)	0.65	0.067(0.410-1.031)
Education (High school or less)	1.52	**0.007**(1.107-2.093)	1.25	0.232(0.865-1.817)

Bold indicates statistically significant p-values under the threshold 0.05
OR = Odds Ratio

4.7 ARTIGO VII:

Manuscrito submetido para publicação no periódico *Cancer Genetics* (QUALIS CAPES A4) em Jan/2024.

GENETIC AND ENVIRONMENTAL FACTORS OF OROFACIAL CLEFT AS MARKERS FOR ORAL POTENTIALLY MALIGNANT DISORDERS AND ORAL CANCER

Abstract

Introduction: This study aimed to investigate the association between orofacial clefts with oral potentially malignant disorders and oral squamous cell carcinoma, focusing on genetic variants and environmental risk factors previously related to these conditions. **Methods:** Case-control study included histologically confirmed oral potentially malignant disorders or oral squamous cell carcinoma cases, and healthy controls matched by age and sex. Genomic DNA was genotyped for rs1533767 (*WNT11*), rs9879992 (*GSK3B*), rs3923087 and rs11867417 (*AXIN2*). Genotype and allele frequency comparisons between the groups were analyzed. Unadjusted and adjusted odds ratios were calculated. **Results:** There were not significant statistical difference between groups regarding family history and orofacial clefts (p=0.52). Three genetic markers – rs1533767 (OR=1.94, p= 0.042, 95%C.I.=1.018-3.694), rs3923087 (or=0.58, p=0.038, 95%C.I.=0.344-0.974), rs11867417 (OR=0.51, p=0.010, 95%C.I.=0.304-0.857) – and some environmental factors were associated with the outcomes. **Conclusions:** Common environmental risks factors and genetic markers for orofacial cleft were associated with oral potential malignant disorders and oral cancer.

Key-words: Orofacial Cleft; Oral Leukoplakia; Lichen Planus; Squamous Cell Carcinoma of Head and Neck; Social Determinants of Health.

Introduction

Orofacial clefts (OC) and oral squamous cell carcinoma (OSCC) are public health problems that can cause aesthetic, functional and emotional implications, contributing to higher morbidity and mortality [1,2]. Both diseases have a multifactorial etiology, characterized by a complex interaction between genetical and environmental factors. These factors can still act as isolated, which makes it difficult to fully understand the etiology of both pathologies [3,4].

Since both OC and cancer have a multifactorial etiology, the hypothesis that these conditions may have a common etiology [5,6] has been suggested in studies that found common Single Nucleotide Polymorphisms (SNPs) simultaneously associated with both conditions [7–9]. WNT is a genetic pathway of cellular growth and differentiation [10]. Genetic alterations in this pathway have been used to explain association between the occurrence of OC and OSCC [11,12], since anomalies in the cell migration, proliferation, differentiation, and apoptosis are closely associated with the occurrence of OC and câncer [13]. Also, several oral lesions characterized as Oral Potentially Malignant Disorders (OPMD) share alterations in molecular mechanisms regulated by these genes [14]. In addition, inequalities in socioeconomic, living and working conditions, and low level of social cohesion increase individuals' susceptibility to environmental factors that can influence their health status through various biological mechanisms leading to disease [15]. Environmental factors, such as smoking and alcohol abuse, are related to a higher risk for OC and cancer [16,17].

The etiological intersection between OC and oral cancer could be suggested by environmental factors capable of modulating gene expression in common biological pathways [18] predisposing to OC, when alterations occur during embryonic development, and to OPMD and/or oral cancer, when they occur later in life. Although that, there are few studies investigating the association between OC with oral câncer [11,12] and OPMD [19]. In this context, the objective of this study is to investigate the association between orofacial clefts and oral potentially malignant disorders and oral squamous cell carcinoma, focusing on genetic variations and environmental risk factors.

Materials and methods

This study included 144 subjects, who sought treatment at the State University of Feira de Santana dental clinics. Cases consisted of 48 subjects with histopathologic diagnosis of OPMD (Leukoplakia and Lichen Planus) or oral cancer (OSCC). Individuals with history of

another type of cancer was excluded. Cases were matched by sex and age in a 1:2 ratio with a comparison group of unrelated subjects without history of any OPMD or oral cancer. These controls were selected from the same reference population as the cases, in order to ensure greater comparability regarding demographic, ethnic and sociocultural characteristics.

All participants provided an informed consent to participate. This study has the approval of State University of Feira de Santana (approval ## 61386322.8.0000.0053) and of the University of Pittsburgh (IRB ## 03829018.1.0000.5183). We followed the STROBE (STrengthening the Reporting of Observational studies in Epidemiology) and STREGA (Trengthening the REporting of Genetic Association Studies) guidelines.

Information regarding biologic and environmental (behavioral/lifestyle, and socioeconomic) factors were obtained by interviewing. All participants were clinically evaluated for tooth agenesis and tooth extraction considering all permanent teeth. For DNA isolation, 4 ml of unstimulated saliva was collected from each participant, followed by genomic DNA extraction using an established protocol. Genotyping was performed blindly to clinical diagnosis status. Reactions were carried out using TaqMan chemistry [20] in the GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA). Applied Biosystems supplied assays and reagents. Endpoint analysis of PCR products was performed using the QuantStudio 6 Flex (Applied Biosystems, Foster City, CA, USA). It was selected for the genotyping four SNPs from three WNT pathway genes that have been previously suggested to be associated with OC and oral cancer [11,12]. They were rs1533767 (WNT11), rs9879992 (GSK3B), rs3923087 and rs11867417 (both in AXIN2). Information of the SNPs was obtained from the NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/snp). PCR reactions were repeated twice when necessary, remaining acceptable missing rates varying between 6.9% (rs11867417) to 12.5% (rs1533767).

The dependent variable was the OPMD/OSCC while the independent variable was family history of OC (for epidemiologic analysis) and the SNPs (for genetic analysis). Both analyzes took into account the same covariates (sex, age, ethnicity, educational level, civil status, consanguinity relationship, occupation, local of residence, familiar history of cancer, history of agenesis, and alcohol and tobacco habits) (Table 1).

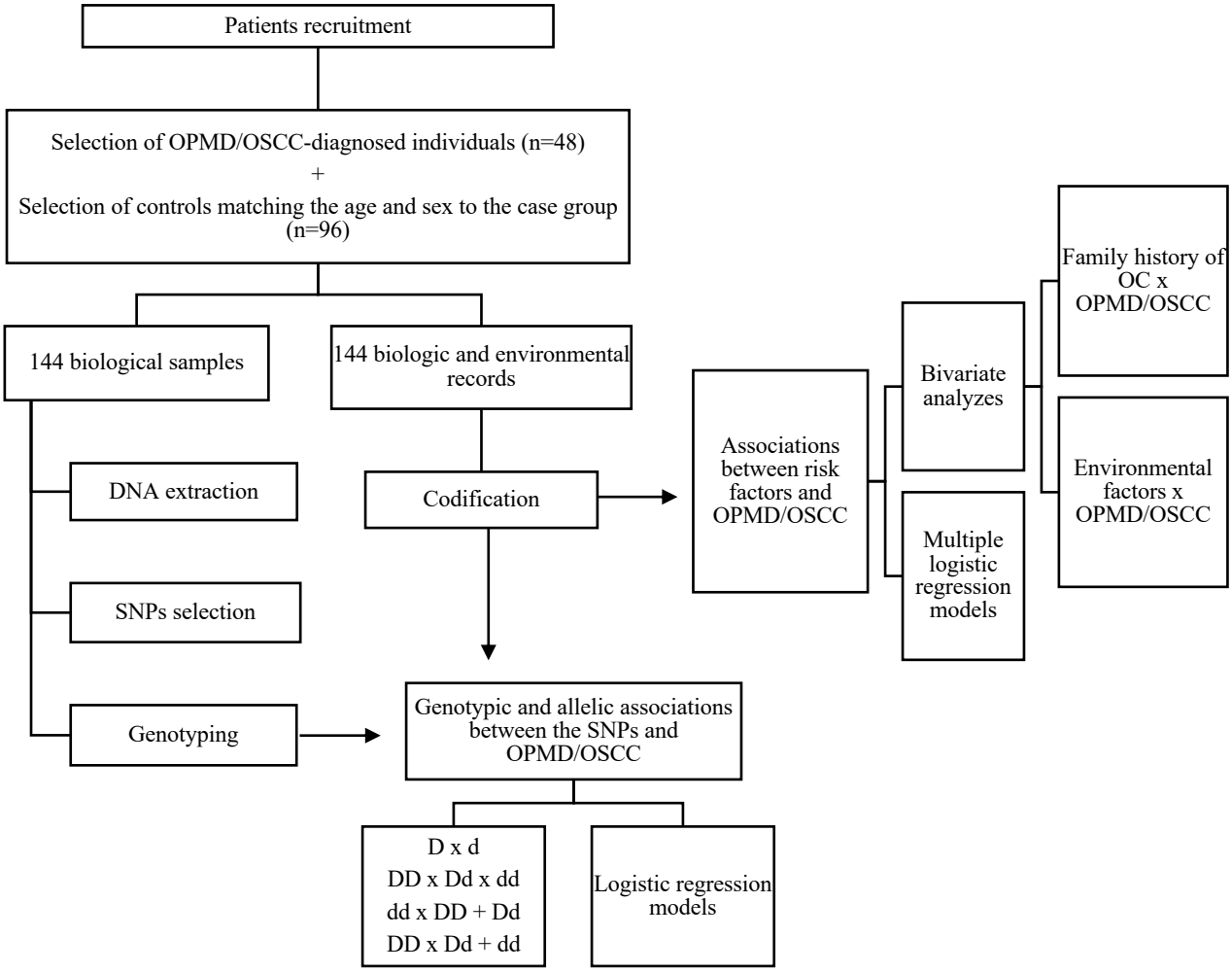
Table 1 – Description of the variables included in the study

Category	Variable	Description
Biologic characteristics	Family history of Orofacial Clefts	Yes, No
	Single polymorphisms nucleotides	rs1533767 (*WNT11*), rs9879992 (*GSK3B*), rs3923087 and rs11867417 (both in *AXIN2*)
	Sex	Female, Male
	Age	(As continuous)
	Ethnicity	White, Pard, Black
	Consanguinity relationship	Yes, No
	Family history of cancer	Yes, No
Behavioral/ lifestyle characteristics	History of dental agenesis/ extraction	Yes, No
	Number of missing teeth	(As continuous)
	Smoking habits	Yes, No
	Type of Smoking	Cigarette, Cigarette and Others, Others
	Alcohol consumption habits	Yes, No
	Type of alcoholic beverage	Fermented, Distillated, Both
Socioeconomic characteristics	Civil Status	Single, Married, Divorced, Widow
	Local of residence	Urban, Rural
	Education	Illiterate, elementary school, High school, Graduate
	Occupation	Agricultural worker, others

For epidemiologic analyzes, the sample was characterized according to clinical, biological and environmental (socioeconomic factors, behavioral and lifestyle habits) features. Chi-square and Fisher exact were used in order to detect differences between cases and controls. Bivariate associations between family history of OC and OPMD/OSCC and between environmental risk factors and OPMD/OSCC were assessed by Odds Ratio (OR). Multiple logistic regression models were performed to obtain the adjusted OR for the previous associations. All analyzes were performed at the STATA (Software for Statistics and Data Science), assuming a p-value below 0.05 and a 95% Confidence Interval (95%I.C.) as statistically significant.

For genetic analyzes, Hardy–Weinberg equilibrium (HWE) was assessed through the chi-square test, assuming p-values above 0.012 (0.05/4; the denominator is the number of genetic markers) as statistically significant. Over representation of genotypes and alleles frequencies in both groups were determined. Considering d as most common allele and D as the less common allele, the genetic models included were the allelic model (D x d), genotypic model (DD x Dd x dd), dominant model (dd x DD + Dd), and recessive model (DD x Dd + dd) [21]. Logistic regression models were performed to obtain the adjusted OR regarding the alleles and outcomes associations. Genetic analyzes were done at Plink Software, assuming a p-value bellow 0.05 and a 95% Confidence Interval (95%I.C.) as statistically significant (Figure 1).

Figure 1 – Overall study design



OPMD: oral potentially malignant disorders;
OSCC: Oral Squamous Cell Carcinoma; SNPs: Single Nucleotide Polymorphisms

Results

Biologic, behavioral/lifestyle, and socioeconomic profile of the study population

The mean age of the individuals included was 60 (±15) years [cases: 60.3 (±15.6); controls: 60.5 (±15); p=0.436)], varying between 22-87 years. The majority of them were male (54.8%), and self-reported as brown (48.6%) (Table 4). Few individuals had history of consanguinity and among those, one was at 3rd degree and nine were at 4th degree. Most of the individuals had family history of cancer. There was not statistical significance difference among cases and controls regarding the biologic characteristics (Table 2).

Both groups reported history of dental extraction (93%). The mean number of missing teeth was statistically significant different between cases and controls (p=0.001), 16 (±12) and

13(±9) respectively. Case individuals were more edentulous while controls tended to have fewer missing teeth (p=0.006) (Table 2). Also, both groups reported have or had had smoking habits (54.9%), however individuals with OPMD/OSCC reported a higher frequency of that(p=0.024). Most individuals consumed or consume alcoholic beverages (65.3%), being fermented (n=52), distillated (n=19), or both (n=20) (Table 2).

Regarding the socioeconomic characteristics, most of the individuals were married (n=72), urban residents (n=115), with elementary school education (n=66), and service/market workers (n=59). With the exception of civil status, all covariates were statistically significant different distributed between cases and controls, suggesting that individuals with OPMD/OSCC seem to live more at rural locations (p=0.000), have less years of school education (p=0.04), and work with agriculture (p=0.006) (Table 2).

Table 2– Descriptive analysis and comparisons of the characteristic obtained from the study population according to the diagnostic groups

		Total Population n(%)	Case n(%)	Control n(%)	p value
Biologic characteristics	Sex				1.000
	Female	69(45.2%)	23(47.9%)	46(47.9%)	
	Male	75(54.8%)	25(52.1%)	50(52.1%)	
	Ethnicity				0.971
	White	25(17.4%)	8(16.7%	17((17.7%)	
	Brown	70(48.6%)	24(50.0%)	46(47.9%)	
	Black	49(34.0%)	16(33.3%)	33(34.3%)	
	Consanguinity relationship				0.854
	No	131(92.9%)	43(93.5%)	88(92.6%)	
	Yes	10(7.1%)	3(6.5%)	7(7.4%)	
	Family history of cancer				0.099
	No	74(51.4%)	20(41.7%)	54(56.2%)	
	Yes	70(48.6%)	28(58.3%)	42(43.7%)	
	Family history of Orofacial Clefts				0.520
	No	139(96.5%)	47(97.9%)	92(95.8%)	
	Yes	5(3.5%)	1(2.1%)	4(4.2%)	
Behavioral/lifestyle characteristics	History of extraction				0.816
	No	10(7.0%)	3(6.3%)	7(9.3%)	
	Yes	134(93.0%)	45(93.7%)	89(92.7%)	
	Number of missing teeth (categoric)				**0.006**
	1-5 teeth	36(25.3%)	11(23.4%)	25(26.4%)	
	6-15 teeth	47(33.1%)	14(29.7%)	33(34.7%)	
	16-31 teeth	44(31.0%)	11(23.4%)	33(34.7%)	
	Edentulous	14(10.6%)	11(23.4%)	4(4.2%)	
	Smoking habits				**0.024**
	No	79(54.9%)	20(41.7%)	59(61.5%)	
	Yes	65(45.1%)	28(58.3%)	37(38.5%)	
	Type of Smoking				**0.032**
	Cigarette	51(77.3%)	18(64.3%)	33(86.8%)	
	Cigarette and other	5(7.6%)	2(7.1%)	3(7.9%)	
	Others	10(15.1%)	8(28.6%)	2(5.26%)	
	Alcohol consumption habits				0.536
	No	50(34.7%)	15(31.2%)	35(36.5%)	

	Yes	94(65.3%)	33(68.3%)	61(63.5%)	
	Type of alcoholic beverage				0.536
	Fermented	52(57.1%)	14(43.7%)	38(64.4%)	
	Distillated	19(20.9%)	11(34.4%)	8(13.6%)	
	Both	20(22.0%)	7(21.9%)	13(22.1%)	
Socioeconomic characteristics	Civil Status				0.686
	Single	39(27.3%)	16(33.3%)	23(24.2%)	
	Married	72(50.3%)	23(47.9%)	49(51.6%)	
	Divorced	25(17.5%)	7(14.6%)	18(18.9%)	
	Widow	7(4.9%)	2(4.2%)	5(5.3%)	
	Local of residence				**0.000**
	Urban	115(80.4%)	25(52.1%)	90(94.7%)	
	Rural	28(19.6%)	23(47.9%)	5(5.3%)	
	Education				**0.040**
	Illiterate	17(12.0%)	11(22.9%)	6(6.4%)	
	Elementary school	66(46.5%)	19(39.6%)	47(50.0%)	
	High School	42(29.5%)	13(27.1%)	29(30.8%)	
	Graduate	17(12.0%)	5(10.4%)	12(12.7%)	
	Occupation				**0.006**
	Agricultural workers	26(18.1%)	17(35.4%)	9(9.4%)	
	Service and market workers	59(40.9%)	17(35.4%)	42(43.8%)	
	Mid-level technicians	7(4.9%)	2(4.2%)	5(5.2%)	
	Workers in the production of industrial good/services	3(2.1%)	0(0.0%)	3(3.1%)	
	Maintenance and construction workers	12(8.3%)	1(2.1%)	11(11.5%)	
	Science professionals	13(9.0%)	3(6.2%)	10)10.4%)	
	Administrative service workers	3(2.1%)	2(4.2%)	1(1.0%)	
	Others	21(14.6%)	6(12.5%)	15(15.6%)	

p value through chi square
Bold indicates statistically significant p-values under the threshold 0.05

Association between OC and OPMD/OSCC

None of the individuals had OC. The minority of them had family history of OC, reporting 2nd (n=2) and 3rd (n=3) and degree of kindship. Chi-square test did not show a significant statistic difference between the groups regarding the presence of family history of OC (p=0.52). The bivariate analysis and logistic regression were not statically significant for this association either (table 3).

Table 3 - Unadjusted and adjusted Odd Ratios between environmental factors and OPMD/OSCC

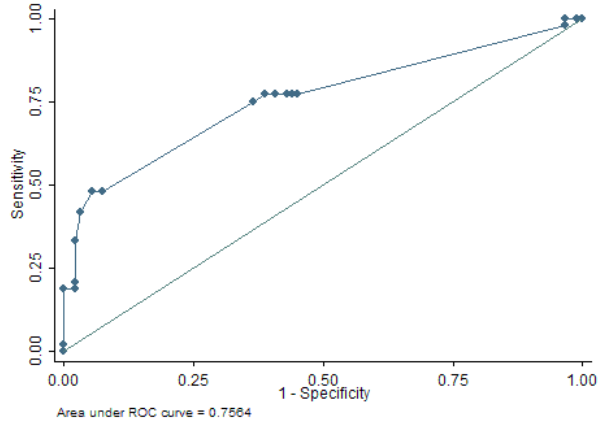
Exposure	OR	p(95%C.I.)	OR (adjusted)	P value (95%C.I.)
Family history of orofacial cleft	0.49	0.528 (0.531-4.0502)	0.88	0.913 (0877-8.804)
Local of residence (rural)	16.56	**0.000** (5.315-59.992)	15.46	**0.000** (3.76-63.543)
Smoking habits (smokers)	2.13	**0.033** (1.056-4.323)	1.92	0.118 (0.847-4.358)
Occupation (agriculture)	5.30	**0.001** (1.970-14.813)	0.77	0.740 (0.176- 3.427)
Education (illiterate)	4.36	**0.004** (1.344-15.309)	1.78	0.419 (0.438-7.264)

OR = Odds Ratio
Bold indicates statistically significant p-values under the threshold 0.05

Association between environmental factors and OPMD/OSCC

The bivariate analysis was performed with the variables that showed a statistically significant different distribution between the groups, giving an OR for each of these associations. However, after multiple logistic regression only local of residence was statistically significant associated (adjusted OR=15.46, p=0.000, 95%C.I.= 3.76-63.543) (Table 3). The multiple logistic model fit was assessed through the goodness of fit test and explanatory power. The Hosmer-Lemeshow (p=0.53) indicated that the model's predicted values are a good match for the real values. The area under ROC curve (ROC=0.756) indicated that the model is good in predict the dependent variable based on the independent variables (Figure 2). The linearity of dichotomy predictors (p=0.676) demonstrated that the non-dichotomous predictors varied linearly with the outcome logit.

Figure 2 – Receiver operating characteristic (ROC) curve logistic regression model for OPMD/OSCC considering, family history of orofacial clefts, local of residence, smoking habits, occupation and educational level



Identification of polymorphisms and analysis of their association with OPMD/OSCC

The genotype distribution was consistent with HWE for the cases for all SNPs - *GSK3B* rs9879992 (p=0.51), *WNT11* rs1533767 (p=0.7), *AXIN2* rs3923087 (p=0.211), and *AXIN2* rs11867417 (p=0.525), meaning that is that there is no significant difference between the observed and the expected genotypic counts under HWE proportions. In the control group, *AXIN2* rs3923087 (p=0.000) showed deviation from HWE.

We found some associations between the phenotypes and SNPs studied (Table 4). The *WNT11* rs1533767 was statistically significant associated with OPMD/OSCC by the genotypic (p = 0.038), allelic (OR = 1.94, p = 0.042, 95%C.I. = 1.018-3.694), and dominant (OR = 1.94, p = 0.042, 95%C.I. = 1.018-3.694) models. The two SNPs in the gene *AXIN2* (rs3923087 and

rs11867417) showed a statistically significant association with OPMD/OSCC by both allelic (OR = 0.58, p = 0.03845, 95%C.I.= 0.344-0.974; and OR=0.51, p=0.010, 95%C.I.= 0.304-0.857, respectively) and recessive models (OR=0.40, p=0.04, 95%C.I. = 0.168-0.975; and OR=0.35, p=0.022, 95%C.I.=0.141-0.883). The *GSK3B* rs9879992 did not present a statistically significant association with OPMD/OSCC by any of the full model tested. The logistic regression models were performed for both *WNT11* (rs1533767) and *AXIN2* (rs3923087 and rs11867417), adjusted by each possible covariate (local of residence, familiar history of cancer, familiar history of orofacial clefts, smoking habits, and alcohol) (Table 5).

Table 4 - Full model association tests

Gene (SNP)	Allele D/d	MAF	Model	Affected	Unaffected	X^2	DF	p value	OR [C.I.95%]
GSK3B (rs9879992)	G/A	0.38	Genotypic	6/18/21	16/36/33	0.999	2	0.607	
			Allele	30/60	68/102	1.114	1	0.291	0.75 [0.273-0.4393]
			Dominant	24/21	52/33	0.745	1	0.427	0.72 [0.349-1.505]
			Recessive	6/39	16/69	0.631	1	0.427	0.66 [0.439-1.281]
WNT11 (rs1533767)	A/G	0.19	Genotypic	2/18/23	4/17/62	6.551	1	**0.038**	
			Allele	22/64	25/141	4.133	1	**0.042**	**1.94 [1.018-3.694]**
			Dominant	20/23	21/62	5.805	1	**0.016**	**2.57 [1.180-5.585]**
			Recessive	2/41	4/79	0.002	1	1.000	0.96 [0.168-0.975]
AXIN2 (rs3923087)	C/T	0.45	Genotypic	8/17/20	31/27/31	4.216	2	0.1215	
			Allele	33/57	89/89	4.285	1	**0.038**	**0.58 [0.344-0.974]**
			Dominant	25/20	58/21	1.172	1	0.279	0.69 [0.321-1.389]
			Recessive	8/37	31/58	4.213	1	**0.040**	**0.40 [0.168-0.975]**
AXIN2 (rs11867417)	C/T	0.47	Genotypic	7/19/20	30/33/26	5.735	2	0.056	
			Allele	33/59	93/85	6.536	1	**0.010**	**0.51 [0.304-0.857]**
			Dominant	26/20	63/26	2.747	1	0.097	0.53 [0.256-1.125]
			Recessive	7/39	30/59	5.211	1	**0.022**	**0.35 [0.141-0.883]**

Allelic model (D x d), genotypic model (DD x Dd x dd), dominant model (dd x DD + Dd), and recessive model (DD x Dd + dd), assuming d as most common allele and D as the less common allele.
MAF: The minor allele frequencies for each SNP, based on all founders in the sample.
Bold indicates statistically significant p-values under the threshold 0.05
X^2 = Chi-square value; OR = Odds Ratio; DF = Degree of freedom

Table 5 - Logistic regression analysis of associations between the SNPs and OPMD/OSCC

SNP/Minor allele	Adjustment	OR	p value
rs1533767/A	Smoking habits	1.75	0.091
	Alcohol consumption	1.84	0.059
	Familiar history of cancer	2.06	**0.029**
	Familiar history of orofacial cleft	1.82	0.659
	Reside in rural areas	1.90	0.067
rs3923087/C	Smoking habits	0.60	**0.036**
	Alcohol consumption	0.65	0.072
	Familiar history of cancer	0.69	0.109
	Familiar history of orofacial cleft	0.64	0.059
	Reside in rural areas	0.66	0.121
rs11867417/C	Smoking habits	0.58	**0.031**
	Alcohol consumption	0.58	**0.026**
	Familiar history of cancer	0.57	**0.024**
	Familiar history of orofacial cleft	0.56	**0.019**
	Reside in rural areas	0.59	**0.050**

Bold indicates statistically significant p-values under the threshold 0.05; OR = Odds Ratio

Discussion

Epidemiology analysis indicated that there was not statistically significant difference regarding family history of OC between the groups, suggesting a lack of association between OC and OPMD/OSCC in the sample studied. Previous studies in this field did not perform epidemiologic analyses [11,12]. Considering the type of cancer, studies reported family history of OC in patients with cancer, but this frequency was not statistically different between groups [8,22,23]. The reasons for that could be related to memory bias [23], or the small sample size [22]. A systematic review revealed an association between cancer and relatives of patients with OC, suggesting more studies to confirm these findings [6].

The most common studied environmental factors for both OC and oral cancer are smoking and alcohol [16,17]. Our results did not find statistically significant association between alcohol and OPMD/OSCC. For smoking habits, it seemed to increase in two times the risk of develop OPMD/OSCC, however this association did not remain after adjustment for confiders.

Tooth agenesis has been associated with OC and cancer [24,25]. We were not able to study this association since the individuals had missing teeth prior the examination. Interesting, we were able to observe a higher frequency of tooth loss/edentulism due to extraction among cases. Since tooth loss/edentulism were not a risk factor in common for both OC and cancer, it was not considered as covariate to enter in the logistic regression analyzes. Instead, more investigations should be done to confirm this finding, taking into account the role of genetics and environmental factors in the susceptibility to dental diseases [26].

Regarding the socioeconomic characteristics, living in rural areas increased in 15 times the risk to have OPMD/cancer. Previous studies found association between rural areas and OC [27–29] or cAncer [30]. A possible explanation is that rural residents are less likely to have health insurance or access to healthcare [28,29].

The genetic investigation focused on potential genetic markers linked to OC and oral cancer to assess their contribution to OPMD/OSCC risk. Our results showed an association between three genetic markers – rs1533767, rs3923087 and rs11867417 – and OPMD/OSCC. These results suggest that variations in WNT genes that were previously related to OC might contribute to an individual's susceptibility to oral dysplasia and OSCC, corroborating with previous evidence of OPMD risk of malignant transformation to cancer [31].

The WNT11 rs1533767 was statistically associated with OPMD/OSCC. The heterozygous genotype (A/G) was more frequent in individuals diagnosed with the diseases.

Also, the presence of at least one copy of the minor allele A was more frequent in the case group, suggesting a risk almost two times higher of individuals with the minor allele A have disease. Similar results were found previously [11]. The dominant model indicated that individuals A/A and A/G were at higher risk for OPMD/OSCC.

Variations in AXIN2 have been associated with cancer, oral leukoplakia, and OC [32–34]. The rs3923087 and rs11867417 (AXIN2) were statistically significant associated with OPMD/OSCC. The presence of at least one copy of the minor allele C was less frequent in cases compared to controls. These SNPs in AXIN2 (rs3923087 and rs11867417) showed a protective effect, since having the minor allele C decreased the risk of OPMD/OSCC. Finally, the recessive models suggested that individuals CC are at lower risk for disease. In another study the allele T for the rs3923087 showed a protective effect for OSCC while the allele T for the rs11867417 was not statically significantly associated [11]. On the same time, an increased abundance of AXIN2 was highly correlated to malignant transformation of oral leukoplakia [34].

The AXIN2 rs3923087 showed a certain deviation from HWE in the control group (p=0.000), as previously reported [35–38]. The reasons for that might be related to unknown population stratification, random mating, and sample bias for this variant [39]. Also, any genotyping error, due to a mistaken allele or allelic dropout, could have caused an increase in homozygotes in individuals (31/27/31). However, the HWE has very low power for detecting genotyping errors, especially when the genotyping error rate is low and the minor allele frequency is not rare. A small population size could also be a reason for the increase in the homozygosity, leading to HWE deviation [40].

The logistic regression models performed between the genetic variants - WNT11 (rs1533767) and AXIN2 (rs3923087 and rs11867417) – and OPMD/OSCC confirmed the allelic associations, showing that family history of cancer and smoking habits seem to work as confounders. It was found association between the rs15337671 in WNT11 (allele A) and an increased risk for OPMD/OSCC, adjusted for family history of cancer. Also, there was association between the SNPs in AXIN2 (allele C) and a decreased risk for OPMD/OSCC adjusted for smoking. Family history of OC, local of residence, and alcohol seem to work as confiders for the association between rs11867417 (allele C) and OPMD/OSCC.

The results of this study need to be considered in light of some limitations. Initially, the relatively small number of patients with OPMD/OSCC, which we tried to minimize by a carefully selection of cases and controls and by the use of two matched controls for each case. Second, we were not able to investigate the association between tooth agenesis, OC and

OPMD/OSCC as explained above. Instead of that, we could assess the association between tooth loss/edentulism and OPMD/OSCC and the result stimulates further investigations.

Genetic and environmental factors play an important role at the etiology of OC and oral cancer [16,41]. In this study we investigated the association between OC and OPMD/OSCC. In order to do it, we studied environmental risk factors and genetic variations that are common related to these phenotypes. This is the first time that both factors were assessed in the same study in order to investigate the etiopathogenesis of OC, OPMD and oral cancer in a Brazilian population.

Conclusion

There was not statistically significant difference regarding family history of orofacial clefts between cases and controls. Even with this lack of association in the sample studied, environmental factors and genetic markers also related to orofacial clefts risk were associated with oral potentially malignant disorders/oral squamous cell carcinoma. These results suggest that variations in WNT pathway genes and exposure to environmental risk factors previously related to orofacial clefts, might modulate individual's susceptibility to oral potentially malignant disorders and/or oral squamous cell carcinoma.

References

[1] Mossey PA, Little J, Munger RG, Dixon MJ, Shaw WC. Cleft lip and palate. The Lancet 2009;374:1773–85. https://doi.org/10.1016/S0140-6736(09)60695-4.

[2] Sung H, Ferlay J, Siegel R, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 2021;71:209–49. https://doi.org/10.3322/caac.21660.

[3] Dixon MJ, Marazita ML, Beaty TH, Murray JC. Cleft lip and palate: synthesizing genetic and environmental influences. NIH Public Access 2011;3:203. https://doi.org/10.1038/nrg2933.Cleft.

[4] Vieira AR. Basis of Inheritance in Humans. Monogr Oral Sci 2022;30:1–19. https://doi.org/10.1159/000520765.

[5] Vieira AR. Cleft Lip/Palate and Cancer: a True Connection. Revista Brasileira de Cancerologia 2021;67:11–3.

[6] Machado RA, Popoff DAV, Martelli-Júnior H. Relationship between non-syndromic oral clefts and cancer: a systematic review and meta-analysis. Oral Dis 2022. https://doi.org/10.1111/odi.14179.

[7] Bui AH, Ayub A, Ahmed MK, Taioli E, Taub PJ. Association Between Cleft Lip and/or Cleft Palate and Family History of Cancer. Ann Plast Surg 2018;80:S178–81. https://doi.org/10.1097/SAP.0000000000001331.

[8] Taioli E, Ragin C, Robertson L, Linkov F, Thurman NE, Vieira AR. Cleft lip and palate in family members of cancer survivors. NIH Public Access 2010;28:958–62. https://doi.org/10.3109/07357907.2010.483510.

[9] Vieira AR, Khaliq S, Lace B. Risk of cancer in relatives of children born with isolated cleft lip and palate. Am J Med Genet A 2012;158 A:1503–4. https://doi.org/10.1002/ajmg.a.35359.

[10] Liu F, Millar SE. Wnt/β-catenin signaling in oral tissue development and disease. J Dent Res 2010;89:318–30. https://doi.org/10.1177/0022034510363373.

[11] Andrade Filho PA, Letra A, Cramer A, Prasad JL, Garlet GP, Vieira AR, et al. Insights from studies with oral cleft genes suggest associations between WNT-pathway genes and risk of oral cancer. J Dent Res 2011;90:740–6. https://doi.org/10.1177/0022034511401622.

[12] Freitas EM de, Machado RA, Santos E de M, Matos FR de, Galvão HC, Soares PBM, et al. Polymorphisms associated with oral clefts as potential susceptibility markers for oral and breast cancer. Arch Oral Biol 2019;99:9–14. https://doi.org/10.1016/j.archoralbio.2018.12.004.

[13] Wang Y, Yang H, Duan G, Wang H. The association of the CYP1A1 Ile462val polymorphism with head and neck cancer risk: Evidence based on a cumulative meta-analysis. Onco Targets Ther 2016;9:2927–34. https://doi.org/10.2147/OTT.S106264.

[14] Ejaz I, Ghafoor S. Wnt Signaling Pathway in Oral Lesions. J Pak Med Assoc 2019;69:1687–92. https://doi.org/10.5455/jpma.5890.

[15] Notterman DA, Mitchell C. Epigenetics and Understanding the Impact of Social Determinants of Health. HHS Public Access 2015;62:1227–40. https://doi.org/doi:10.1016/j.pcl.2015.05.012.

[16] Vieira AR. Genetic and Environmental Factors in Human Cleft Lip and Palate. vol. 16. 2012.

[17] Sarode G, Maniyar N, Sarode SC, Jafer M, Patil S, Awan KH. Epidemiologic aspects of oral cancer. Disease-a-Month 2020;66:100988. https://doi.org/10.1016/j.disamonth.2020.100988.

[18] Cruz LA. Mecanismos genéticos e epigenéticos na etiologia das fissuras orofaciais. Instituto de Biociências da Universidade de São Paulo, 2017.

[19] Bezamat M, Harrison B, Zhou Y, Glickman KM, Telles V, Guirguis C, et al. Phenome-Wide Scan Finds Potential Orofacial Risk Markers for Cancer. Sci Rep 2020;10:1–10. https://doi.org/10.1038/s41598-020-61654-3.

[20] Ranade K, Chang MS, Ting CT, Pei D, Hsiao CF, Olivier M, et al. High-throughput genotyping with single nucleotide polymorphisms. Genome Res 2001;11:1262–8. https://doi.org/10.1101/gr.157801.

[21] Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, et al. PLINK: A tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;81:559–75. https://doi.org/10.1086/519795.

[22] Martelli DRB, Vieira AR, Fonseca AT, Coletta R Della, Soares PBM, Martelli-Júnior H. Risk of nonsyndromic cleft lip and palate in relatives of women with breast cancer. Am J Med Genet A 2014;164:270–1. https://doi.org/10.1002/ajmg.a.36186.

[23] Dias VO, Martelli DRB, Santos ML, Maia CF, De Andrade RS, Martelli-Júnior H. Nonsyndromic oral cleft in first-degree relatives of patients with acute lymphoblastic leukemia. Dent J (Basel) 2020;8:6–11. https://doi.org/10.3390/dj8010023.

[24] Letra A, Menezes R, Granjeiro JM, Vieira AR. AXIN2 and CDH1 polymorphisms, tooth agenesis, and oral clefts. Birth Defects Res A Clin Mol Teratol 2009;85:169–73. https://doi.org/10.1002/bdra.20489.

[25] Küchler EC, Lips A, Tannure PN, Ho B, Costa MC, Granjeiro JM, et al. Tooth agenesis association with self-reported family history of cancer. J Dent Res 2013;92:149–55. https://doi.org/10.1177/0022034512468750.

[26] Upadhyaya C, Humagain M. The pattern of tooth loss due to dental caries and periodontal disease among patients attending dental department (OPD), Dhulikhel Hospital, Kathmandu University Teaching Hospital (KUTH), Nepal. Kathmandu University Medical Journal 2009;7:59–62. https://doi.org/10.3126/kumj.v7i1.1767.

[27] Vieira AR, Furtado PGC, Lacerda Filho VV, Pereira JAT, Tenório IS, Luna AM, et al. Orofacial Cleft Frequency Differences Depending on Geographic Origin: Coast vs. Inland. Frontiers in Dental Medicine 2021;2:1–6. https://doi.org/10.3389/fdmed.2021.670948.

[28] Messer LC, Luben TJ, Mendola P, Carozza SE, Horel SA, Langlois PH. Urban-Rural Residence and the Occurrence of Cleft Lip and Cleft Palate in Texas, 1999-2003. Ann Epidemiol 2010;20:32–9. https://doi.org/10.1016/j.annepidem.2009.09.006.

[29] Kapos FP, White LA, Schmidt KA, Hawes SE, Starr JR. Risk of non-syndromic orofacial clefts by maternal rural-urban residence and race/ethnicity: A population-based case-control study in

Washington State 1989-2014. Paediatr Perinat Epidemiol 2021;35:292–301. https://doi.org/10.1111/ppe.12727.

[30] Rylands J, Lowe D, Rogers SN. Outcomes by area of residence deprivation in a cohort of oral cancer patients: Survival, health-related quality of life, and place of death. Oral Oncol 2016;52:30–6. https://doi.org/10.1016/j.oraloncology.2015.10.017.

[31] Shearston K, Fateh B, Tai S, Hove D, Farah CS. Malignant transformation rate of oral leukoplakia in an Australian population. Journal of Oral Pathology & Medicine 2019;48:530–7. https://doi.org/10.1111/jop.12899.

[32] Li X, Li Y, Liu G, Wu W. New insights of the correlation between AXIN2 polymorphism and cancer risk and susceptibility: evidence from 72 studies. BMC Cancer 2021;21:1–15. https://doi.org/10.1186/s12885-021-08092-0.

[33] Li S, Wang C, Liu X, Hua S, Liu X. The roles of AXIN2 in tumorigenesis and epigenetic regulation. Fam Cancer 2015;14:325–31. https://doi.org/10.1007/s10689-014-9775-7.

[34] Zhang X, Kim KY, Zheng Z, Kim HS, Cha IH, Yook JI. Snail and Axin2 expression predict the malignant transformation of oral leukoplakia. Oral Oncol 2017;73:48–55. https://doi.org/10.1016/j.oraloncology.2017.08.004.

[35] Bahl C, Sharma S, Singh N, Behera D. Association study between genetic variations in Axin2 gene and lung cancer risk in North Indian population: A multiple interaction analysis. Tumor Biology 2017;39. https://doi.org/10.1177/1010428317695533.

[36] Hernández-Arenas YY, Támara-De Ávila JJ, Isaza-Guzmán DM, González-Pérez L V., Tobón-Arroyave SI. Relationship of the XRCC1 rs25487 polymorphism with demographic, behavioral, clinical, and histological parameters in oral potentially malignant disorders and oral squamous cell carcinoma in a Colombian population. J Oral Biosci 2021;63:217–23. https://doi.org/10.1016/j.job.2021.02.006.

[37] Assis IO, Lacerda RHW, Cavalcante BGN, Bezamat M, Modesto A, Vieira AR. IRE1 Less Common Homozygous Genotype in Families with Positive History of Cancer and Individuals Born with Cleft Lip/Palate. Journal of Craniofacial Surgery 2021;32:82. https://doi.org/10.1097/SCS.0000000000007169.

[38] Prescott NJ, Malcolm S. Folate and the Face: Evaluating the Evidence for the Influence of Folate Genes on Craniofacial Development. The Cleft Palate-Craniofacial Journal 2002;39:327–31. https://doi.org/10.1597/1545-1569_2002_039_0327_fatfet_2.0.co_2.

[39] Waples RS, Allendorf F. Testing for hardy-weinberg proportions: Have we lost the plot? Journal of Heredity 2015;106:1–19. https://doi.org/10.1093/jhered/esu062.

[40] Elston RC. Statistical Human Genetics - Methods and Protocols. vol. 2. Cleveland, Ohio: n.d. https://doi.org/10.1007/978-1-4939-7274-6.

[41] Sankpal UT, Pius H, Khan M, Shukoor MI, Maliakal P, Lee CM, et al. Environmental factors in causing human cancers: Emphasis on tumorigenesis. Tumor Biology 2012;33:1265–74. https://doi.org/10.1007/s13277-012-0413-4.

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ORIGINAL ARTICLE

WILEY

Genetic and environmental contributions for the relationship between tooth loss and oral potentially malignant disorders and oral squamous cell carcinoma

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Abstract

Background: To investigate the association between tooth loss and oral potentially malignant disorders and oral squamous cell carcinoma, focusing on epidemiological factors and genetic variants.

Methods: Case–control study, including histologically confirmed oral potentially malignant disorders and oral squamous cell carcinoma cases and healthy controls. Unadjusted and adjusted odds ratios for this association were calculated. Single-nucleotides polymorphisms were tested for individuals with and without missing teeth.

Results: Case individuals were more edentulous while controls had fewer missing teeth ($p = 0.006$). There was an increased risk for the outcomes associated with edentulism (OR = 6.95, $p = 0.000$), even after adjustments for educational level (OR = 4.7, $p = 0.034$) and smoking habits (OR = 5.01, $p = 0.022$). Among individuals with tooth loss, rs1533767 (*WNT11*), rs3923087, and rs11867417 (*AXIN2*) were associated with the outcomes (OR = 1.67, $p = 0.03$, OR = 0.53, $p = 0.05$, and OR = 0.42, $p = 0.00$, respectively).

Conclusions: Tooth loss could increase the risk for oral potentially malignant disorders and oral squamous cell carcinoma.

KEYWORDS

cleft palate, oral leukoplakia, orofacial cleft, squamous cell carcinoma of head and neck, tooth loss

1 | INTRODUCTION

Tooth loss is a condition that causes psychological, social, and functional impacts, leading to a diminished quality of life, reduced social interaction, and limited food choices.[1] It is a common outcome, especially in countries where dental care is less accessible, and dental extraction becomes an option for a significant portion of the population facing tooth pain.[2] The estimated global average prevalence of complete tooth loss is almost 7% among people aged 20 years or older, increasing to 23% for people aged 60 years or older.[1]

The major reason for tooth loss is dental extraction due to advanced dental caries or periodontitis,[3,4] both of which are chronic bacterial infections. Dental caries is caused by gram-positive facultative bacteria that produce lactic acid from the fermentation of carbohydrates, resulting in demineralization of teeth.[5] Periodontal disease is characterized as chronic inflammation of structures around the teeth caused by gram-negative anaerobic

bacteria, leading to alveolar bone loss.[6] Interestingly, specific oral bacteria have also been suggested to be involved in carcinogenesis, increasing the chance of cancer.[7] The relationship between these conditions is based on the ability of these pathogenic bacteria to maintain a constant chronic infection and the systemic inflammatory response.[7–10] They can also directly affect specific intracellular pathways, activate oncogenic pathways, reduce proapoptotic protein expression, and increase cell migration and invasion.[9] These alterations at the cellular level can furthermore induce permanent genetic alterations in epithelial cells,[8] triggering abnormal cell divisions and eventually even carcinoma development.[11]

Results have shown that patients suffering from periodontitis have a two to five times higher risk for cancer, even among patients who have never smoked.[11,12] Additionally, results observed that periodontitis was associated with an increased risk of oral potentially malignant disorders (OPMD).[13] These pieces of evidence strengthen the hypothesis that tooth loss due to dental diseases may be associated with an increased risk for several cancers,[14,15] such as oral squamous cell carcinoma (OSCC).[16] It was observed that an increase of 10 in tooth loss was associated with a 31% increment in head and neck cancer risk.[15] Thus, an increased number of missing teeth, as a sign of periodontitis, has been linked to a higher OSCC prevalence.[10] Genetic studies have identified some single-nucleotide polymorphisms (SNPs) that can contribute to cancer risk in patients with tooth loss due to dental diseases.[14,17] However, the evidence of a relationship between tooth loss and oral cancer still is not completely understood.[16,18]

Considering the roles of genetics[19] and environmental factors[4] in the susceptibility to tooth loss due to both dental caries and periodontal disease, further investigations using variations in genes commonly related to these oral conditions can help elucidate the relationship between tooth loss/edentulism and oral cancer. Identifying tooth loss/edentulism as an oral health outcome related to OPMD/cancer risk could positively impact cancer outcomes and survival rates with the timely implementation of preventive and diagnostic measures.[14] In this context, the objective of this study is to investigate the association between tooth loss and oral potentially malignant disorders and oral squamous cell carcinoma, focusing on epidemiological factors and single-nucleotide polymorphisms related to these conditions in individuals from the Brazilian population.

2 | MATERIALS AND METHODS

This is a case–control study developed with a dataset consisting of clinical information linked to DNA samples from 144 subjects, who sought treatment at the State University of Feira de Santana dental clinics. Cases were recruited from the oral pathology clinic, while controls were recruited from general dental clinics, both situated at this institution, in order ensure comparability in their behavioral and socioeconomic characteristic (Figure 1).

The cases consisted of 48 subjects with histopathologic diagnosis of OPMD (12 with oral leukoplakia and 15 with oral lichen planus) or oral squamous cell carcinoma (OSCC; 16 with oral cancer). Individuals who have been treated for these diseases in the past or considered to be in remission for at least 6 months, or who are being followed up will also be eligible as case for the study. However, individuals with a history of cancer in an anatomical location other than the oral cavity and oropharynx were excluded.

Controls were matched by sex and age (age ± 5 years), in a 1:2 ratio. Individuals with a history of cancer or potentially malignant disorders, as well as related or family members of those eligible for the case group, were excluded from the control group. This exclusion aimed to mitigate the potential bias in the study arising from shared habits influenced by social interactions.

All participants completed a written document detailing the purpose of the study and provided informed consent to participate. This study has the Institutional Review Board approval of the State University of Feira de Santana (approval ## 61386322.8.0000.0053) and the University of Pittsburgh Institutional Review Board (IRB ## 03829018.1.0000.5183), and it was reported according to the recommended STROBE (STrengthening the Reporting of Observational studies in Epidemiology) statement.[20] All methods were performed in accordance with regulations, and we followed the Strengthening the Reporting of Genetic Association Studies (STREGA) guidelines for this report.[21]

All individuals were clinically evaluated for tooth loss, considering all permanent teeth (32 teeth as a reference). The number of missing teeth for each subject was calculated by subtracting the total number of teeth in the mouth from 32 teeth. After this calculation, the variable was categorized as follows: loss of ≤5 teeth (reference group), loss of 6–15 teeth, loss of 16–31 teeth, and edentulism (32 teeth lost).[16,18,22] Information regarding the reason for tooth loss and potential biological and environmental (behavioral/lifestyle and socioeconomic) risk factors were obtained by interviewing the patients using a questionnaire.

For DNA isolation, 4 mL of unstimulated whole saliva were collected from each participant into a 50-mL sterile plastic container, followed by genomic DNA extraction using an established protocol. After that, genotyping was performed blindly to clinical diagnosis status.

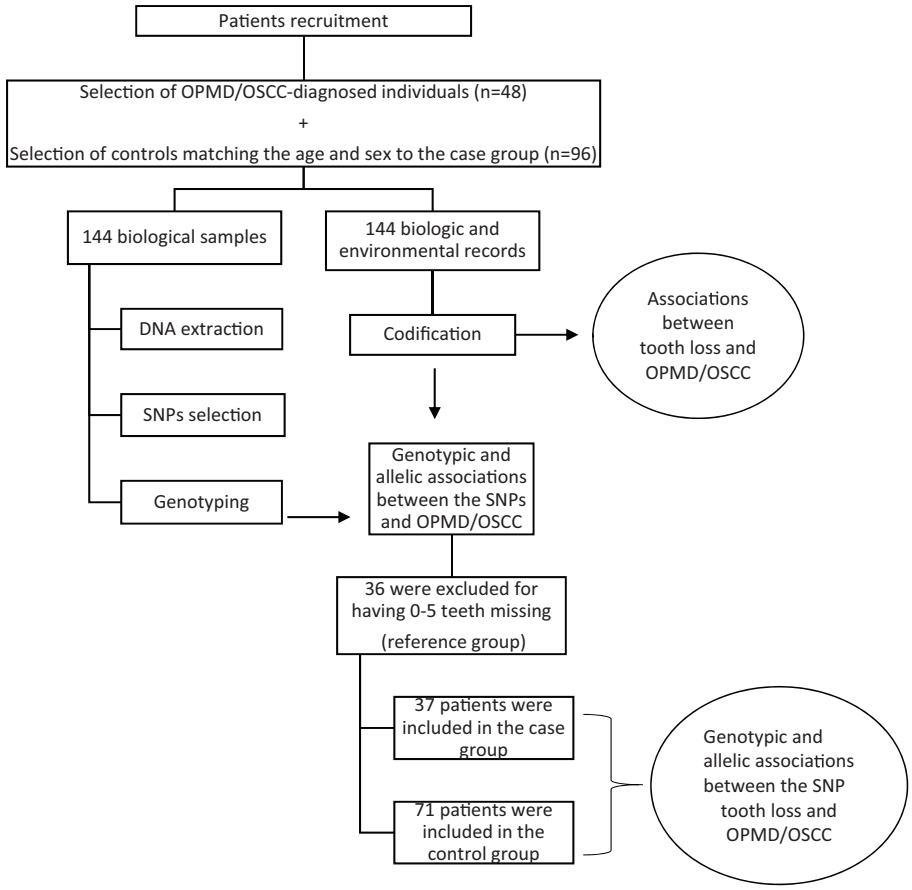

FIGURE 1 Overall study design. OPMD, oral potentially malignant disorders.

Reactions were carried out using TaqMan chemistry[23] in the GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA). Applied Biosystems supplied assays and reagents. Endpoint analysis of PCR products was performed using the QuantStudio 6 Flex (Applied Biosystems, Foster City, CA). PCR reactions were repeated twice when necessary.

For genotyping, five single-nucleotide polymorphisms (SNPs) were selected from three WNT pathway genes—WNT11 (rs1533767), GSK3B (rs9879992), and AXIN2 (rs3923087, rs2240308, and rs11867417), based on their associations with both oral cancer[24,25] and overall cancer[26] as well as craniofacial phenotypes such as oral leukoplakia,[27,28] tooth loss,[27] orofacial clefts, and tooth agenesis.[29,30] Additionally, the SNP rs196929 in ERN1 was selected due to its association with dental caries, periodontitis, and periapical lesions in previous studies.[14,17,31] These SNPs are presented in pathways involved in cell proliferation, differentiation, and inflammation and may contribute to OPMD/OSCC risk as well.[32–35] Information on the six SNPs (variant type, alleles, functional consequence, and minor allele frequency) was obtained from the NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/snp). The ALFA project provides a global aggregate allele frequency from dbGaP (Table 1).

For epidemiologic analyses, it was considered OPMD/OSCC as dependent variable and tooth loss as independent variable. The sample was characterized according to clinical, biological, and environmental (socioeconomic factors, behavioral and lifestyle habits) features. The means and corresponding standard deviations (SDs) were used to summarize the continuous variables, whereas percentages were used to summarize the binary variables. Chi-square and Fisher exact were used in order to detect differences between cases and controls. The association between the number of missing teeth and OPMD/OSCC was also evaluated. Bivariate association between tooth loss and OPMD/OSCC were assessed by odds ratio (OR). Multiple logistic regression model was performed to obtain the adjusted OR, considering covariates that showed to be associated to OPMD/OSCC. All analyses were performed at the STATA software, assuming a *p*-value below 0.05 and a 95% confidence interval (95%CI) as statistically significant.

The genetic analyses were first performed with the total sample, to investigate genetic association with the candidate genes, and after the sample was divided in those without tooth loss (considered as the reference group for tooth loss), and those with individuals with tooth loss (>6 teeth lost) (Figure 1). Hardy–Weinberg

TABLE 1 Summary of candidate genes and SNPs studied.

Gene	Chromosome	SNP	SNP loci	Base change	Minor allele (MAF)
GSK3B	03	rs9879992	Intron	A > G	G (0.25)
WNT11	*11*	*rs1533767*	*Exon*	*G > A*	*A (0.24)*
AXIN2	17	rs11867417	Intron	T > C	T (0.39)
AXIN2	17	rs3923087	Intron	T > C	T (0.27)
AXIN2	17	rs2240308	Missense	G > A	A (0.49)
ERN1	17	rs196929	Intron	T > C, A, G	T (0.27)

Abbreviation: MAF, minor allele frequency (from dbGaP).

TABLE 2 Descriptive analysis and comparisons of the characteristic obtained from the study population according to the diagnostic groups.

		Total population	Case	Control	
		***n* (%)**	***n* (%)**	***n* (%)**	***p*-value**
Biologic characteristics	Sex				1.000
	Female	69 (45.2%)	23 (47.9%)	46 (47.9%)	
	Male	75 (54.8%)	25 (52.1%)	50 (52.1%)	
Behavioral/lifestyle characteristics	Smoking habits				**0.024**
	No	79 (54.9%)	20 (41.7%)	59 (61.5%)	
	Yes	65 (45.1%)	28 (58.3%)	37 (38.5%)	
	Type of smoking				**0.032**
	Cigarette	51 (77.3%)	18 (64.3%)	33 (86.8%)	
	Cigarette and other	5 (7.6%)	2 (7.1%)	3 (7.9%)	
	Others	10 (15.1%)	8 (28.6%)	2 (5.26%)	
	Alcohol consumption habits				0.536
	No	50 (34.7%)	15 (31.2%)	35 (36.5%)	
	Yes	94 (65.3%)	33 (68.3%)	61 (63.5%)	
	Type of alcoholic beverage				0.536
	Fermented	52 (57.1%)	14 (43.7%)	38 (64.4%)	
	Distillated	19 (20.9%)	11 (34.4%)	8 (13.6%)	
	Both	20 (22.0%)	7 (21.9%)	13 (22.1%)	
Socioeconomic characteristics	Ethnicity				0.971
	White	25 (17.4%)	8 (16.7%	17 (17.7%)	
	Brown	70 (48.6%)	24 (50.0%)	46 (47.9%)	
	Black	49 (34.0%)	16 (33.3%)	33 (34.3%)	
	Local of residence				**0.000**
	Urban	115 (80.4%)	25 (52.1%)	90 (94.7%)	
	Rural	28 (19.6%)	23 (47.9%)	5 (5.3%)	
	Education				**0.040**
	Illiterate	17 (12.0%)	11 (22.9%)	6 (6.4%)	
	Elementary school	66 (46.5%)	19 (39.6%)	47 (50.0%)	
	High school	42 (29.5%)	13 (27.1%)	29 (30.8%)	
	Graduate	17 (12.0%)	5 (10.4%)	12 (12.7%)	

Note: *p*-value through chi-square. Bold indicates statistically significant *p*-values under the threshold 0.05.

equilibrium (HWE) was assessed to all sample through the chi-square test, assuming *p*-values above 0.008 (0.05/6; the denominator is the number of genetic markers) as statistically significant. Over representation of genotypes and alleles in affected subjects in comparison to the unaffected matched individuals were determined. Considering d as most common allele and D as the less common allele, the genetic models included were the allelic model (D × d), the genotypic model (DD × Dd × dd), dominant model (dd × DD + Dd), and recessive model (DD × Dd + dd).[14] Logistic regression models were performed to obtain the adjusted OR regarding the alleles and outcomes associations. Genetic analyses were done at Plink Software, assuming a *p*-value bellow 0.05 and a 95%CI as statistically significant, and Bonferroni correction for multiple testing.

3 | RESULTS

The mean age of the individuals included in this study was 60 (±15). The majority of them were male (54.8%), and self-reported as brown (48.6%). There was no statistically significant difference among cases and controls regarding these characteristics (Table 1). Behavioral/lifestyle and socioeconomic potential covariates are presented in Table 2, showing that the local of residence, smoking habits, and education were statistically significantly associated with OPMD/OSCC.

Most of the population (93%) had a history of tooth loss—44 (91.7%) and 88 (90.6%) among cases and controls, respectively, $p = 0.837$—self-reported due to tooth extraction as a result of some dental disease (such as caries or periodontal disease). The mean number of missing teeth was statistically significantly different between cases and controls ($p = 0.001$), being 16 (±12) and 13 (±9), respectively. After categorization, it was found that case individuals showed to be more edentulous while controls tended to have fewer missing teeth ($p = 0.006$). The bivariate analysis performed showed an increased risk for OPMD/OSCC associated with edentulism (OR = 6.95, $p = 0.000$, 95%CI = 1.874–31.418). Results for the logistic regression, adjusted by smoking habits, education, and by both, are shown in Table 3.

The genotype distribution was consistent with HWE for the case groups for all SNPs—GSK3B rs9879992 ($p = 0.510$), WNT11 rs1533767 ($p = 0.700$), AXIN2 rs3923087 ($p = 0.211$), AXIN2 rs11867417 ($p = 0.525$), AXIN2 rs2240308 ($p = 0.291$), ERN1 rs196929 ($p = 0.008$)—meaning that there is no significant difference between the observed and the expected genotypic counts under Hardy–Weinberg proportions. In the control group, the variants AXIN2 rs3923087 ($p = 0.000$) and rs2240308 showed a certain deviation from HWE (Table 4).

The full model association tests make it possible to perform tests of association between OPMD/OSCC and the genetic variants tested. Assuming that D is the minor allele and d is the common allele, the genotypic model is an additive two-degree-of-freedom model that compares the frequencies of each genotype in the groups

TABLE 3 Association of oral potentially malignant disorders/oral squamous cell carcinoma with tooth loss.

No. of lost teeth	Case	Control	*p*-value[a]	Unadjusted OR, *p*-value (95%CI)	Adjusted OR[b], *p*-value (95%CI)	Adjusted OR[c], *p*-value (95%CI)	Adjusted OR[d], *p*-value (95%CI)
≤5 teeth	11 (23.4%)	25 (26.4%)	**0.006**	Ref [1.00]	Ref [1.00]	Ref [1.00]	Ref [1.00]
6–15 teeth	14 (29.8%)	33 (34.7%)		0.80 0.555 (0.344–1.792)	0.84 0.728 (0.315–2.239)	0.86 0.755 (0.324–2.263)	0.74 0.574 (0.274–2.049)
16–31 teeth	11 (23.4%)	33 (34.7%)		0.57 0.169 (0.233–1.345)	0.68 0.447 (0.246–1.855)	0.68 0.454 (0.247–1.867)	0.610 0.350 (0.217–1.718)
32 teeth (edentulous)	11 (23.4%)	4 (4.2%)		6.95 **0.000** (1.874–31.418)	4.70 **0.034** (1.125–19.60)	5.01 **0.022** (1.266–19.83)	3.77 0.074 (0.878–16.20)
Losing teeth, per tooth	16.2 (12.0)	13.1 (9.0)	**0.001**	1.02 0.097 (0.994–1.066)	1.02 0.239 (0.985–1.060)	1.02 0.198 (0.987–1.061)	1.01 0.398 (0.978–1.055)

Note: Bold indicates statistically significant *p*-values under the threshold 0.05.

[a]*p*-value through Pearson chi-square test or *t* test.

[b]Adjusted for smoking.

[c]Adjusted for education.

[d]Adjusted for smoking and education.

TABLE 4 Hardly Weinberg equilibrium (HWE) discrimination of each polymorphic site.

Gene (SNP)	Allele D/d	Group	Observed heterozygotic	Expected heterozygotic	HWE (*p*-value)
GSK3B (rs9879992)	G/A	Affected	0.40	0.44	**0.510**
		Unaffected	0.42	0.48	**0.267**
WNT11 (rs1533767)	A/G	Affected	0.41	0.38	**0.700**
		Unaffected	0.20	0.25	**0.078**
AXIN2 (rs11867417)	C/T	Affected	0.41	0.46	**0.525**
		Unaffected	0.37	0.50	**0.019**
AXIN2 (rs3923087)	C/T	Affected	0.37	0.46	**0.211**
		Unaffected	0.30	0.50	0.000
AXIN2 (rs2240308)	C/T	Affected	0.34	0.40	**0.291**
		Unaffected	0.25	0.41	0.000
ERN1 (196929)	C/T	Affected	0.30	0.49	**0.008**
		Unaffected	0.40	0.49	**0.120**

Note: Bold indicates statistically significant *p*-values above the threshold 0.008 (0.05/6; the denominator is the number of genetic markers) was considered as statistically significant. D is the minor allele and d is the common allele.

(DD × Dd × dd), providing a general test of association of disease by genotype. The dominant and recessive models are tests for the minor allele. The dominant model compares the two copies of the common allele frequency versus the other combinations (DD, Dd vs. dd), and the recessive model compares the two copies of the minor allele frequency versus the other combinations (DD vs. Dd, dd). The trend is the Cochran–Armitage test that does not assume Hardy–Weinberg equilibrium. The allelic model compares the frequencies of each allele in each group (D × d).[36] We found some associations between the outcome (OPMD/oral cancer) studied and polymorphisms in WNT genes. However, the associations were no longer significant after Bonferroni correction for multiple tests. Table 5 summarizes the results for associations between SNPs and OPMD/OSCC.

When analyzing individuals with and without tooth loss separately, the results showed that WNT11 (rs1533767) and AXIN2 (rs3923087 and rs11867417) were statistically associated with OPMD/OSCC in those with tooth loss (Table 6). The presence of at least one copy of the A allele for rs1533767 (WNT11) was more frequent in individuals with tooth loss diagnosed with OPMD/OSCC compared to the controls (28% vs. 16%), suggesting a dominant genetic model (OR = 1.67, $p = 0.03$). For AXIN2 (rs3923087 and rs11867417), the presence of at least one copy of the C allele was less frequent in individuals with tooth loss diagnosed with OPMD/OSCC (OR = 0.53, $p = 0.05$ and OR = 0.42, $p = 0.00$, respectively) than controls. For rs11867417 (AXIN2), the rare homozygous genotype (CC) was less frequent in those with tooth loss diagnosed with OPMD/OSCC compared to the controls (16% vs. 37%), suggesting a recessive genetic model (OR = 0.36, $p = 0.04$). After logistic regressions, associations between rs11867417 (AXIN2) and OPMD/OSCC in those with tooth loss remained statistically significant (Table 7). No significant associations between these SNPs and OPMD/OSCC were identified among individuals without tooth loss (Chart 3).

4 | DISCUSSION

Periodontal disease and dental caries are bacterial infections commonly associated with tooth loss in adults.[3,4] Since oral bacteria implicated in the pathogenesis of these diseases have been suggested to play a role in carcinogenesis,[7–9] studies have focused on investigating whether tooth loss increases the risk of cancer.[10,14–16,18]

The results of this study showed that edentulism increased the risk for OPMD/OSCC (OR = 6.95, $p = 0.000$, 95%CI = 1.874–31.418), even after individual adjustments for educational level and smoking habits (OR = 6.95, $p = 0.000$, 95%CI = 1.874–31.418; OR = 4.70, $p = 0.034$, 95%CI = 1.125–19.60; and OR = 5.01, $p = 0.022$, 95%CI = 1.266–19.83, respectively). However, when this association was adjusted for both covariates simultaneously, the result lost significance. Additionally, the analysis per tooth lost did not show a significant increase in the risk for OPMD/OSCC.

Genetic factors also play an important role in the development of oral cancer[37]; hence, we included the genetic background of participants in our study. The genetic results showed that three of the SNPs tested here were associated with OPMD/OSCC risk. The WNT11 (rs1533767) was associated by the genotypic ($p = 0.038$),

TABLE 5 Full model association tests.

Gene (SNP)	Allele D/d	MAF	Model	Affected	Unaffected	χ^2	DF	*p*-value	OR [95%CI]
GSK3B (rs9879992)	G/A	0.38	Genotypic	6/18/21	16/36/33	0.999	2	0.607	
			Trend	30/60	68/102	0.998	1	0.318	
			Allele	30/60	68/102	1.114	1	0.291	0.75 [0.273–0.4393]
			Dominant	24/21	52/33	0.745	1	0.427	0.72 [0.349–1.505]
			Recessive	6/39	16/69	0.631	1	0.427	0.66 [0.439–1.281]
WNT11 (rs1533767)	A/G	0.19	Genotypic	2/18/23	4/17/62	6.551	1	**0.038**	
			Trend	22/64	25/141	3.811	1	0.051	
			Allele	22/64	25/141	4.133	1	**0.042**	**1.94 [1.018–3.694]**
			Dominant	20/23	21/62	5.805	1	**0.016**	**2.57 [1.180–5.585]**
			Recessive	2/41	4/79	0.002	1	1.000	0.96 [0.168–0.975]
AXIN2 (rs3923087)	C/T	0.45	Genotypic	8/17/20	31/27/31	4.216	2	0.1215	
			Trend	33/57	89/89	3.203	1	0.073	
			Allele	33/57	89/89	4.285	1	**0.038**	**0.58 [0.344–0.974]**
			Dominant	25/20	58/21	1.172	1	0.279	0.69 [0.321–1.389]
			Recessive	8/37	31/58	4.213	1	**0.040**	**0.4 [0.168–0.975]**
AXIN2 (rs11867417)	C/T	0.47	Genotypic	7/19/20	30/33/26	5.735	2	0.056	
			Trend	33/59	93/85	5.331	1	**0.020**	
			Allele	33/59	93/85	6.536	1	**0.010**	**0.51 [0.304–0.857]**
			Dominant	26/20	63/26	2.747	1	0.097	0.53 [0.256–1.125]
			Recessive	7/39	30/59	5.211	1	**0.022**	**0.35 [0.141–0.883]**
AXIN2 (rs2240308)	C/T	0.29	Genotypic	5/16/26	16/23/53	1.874	2	0.391	
			Trend	26/68	55/129	0.113	1	0.736	
			Allele	26/68	55/129	0.150	1	0.698	0.89 [0.517–1.556]
			Dominant	21/26	39/53	0.066	1	0.796	1.1 [0.541–2.229]
			Recessive	5/52	16/76	1.106	1	0.292	0.565 [0.193–1.653]
ERN1 (196929)	C/T	0.44	Genotypic	14/14/18	20/34/30	1.404	2	0.495	
			Trend	42/50	74/94	0.049	1	0.824	
			Allele	42/50	74/94	0.061	1	0.803	1.06 [0.640–1.779]
			Dominant	28/18	54/30	0.149	1	0.699	0.86 [0.412–1.814]
			Recessive	14/32	20/64	0.675	2	0.411	1.4 [0.627–3.128]

Note: Bold indicates statistically significant *p*-values under the threshold 0.05. Allelic model (D × d), genotypic model (DD × Dd × dd), dominant model (dd × DD + Dd), and recessive model (DD × Dd + dd), assuming d as most common allele and D as the less common allele.
Abbreviations: MAF, the minor allele frequencies for each SNP, based on all founders in the sample; χ^2, chi-square value; OR, odds ratio; DF, degrees of freedom.

allelic ($p = 0.042$), and dominant ($p = 0.016$) models, while SNPs in the gene AXIN2 (rs3923087 and rs11867417) were statistically significantly associated by allelic and recessive models. When analyzing individuals with and without tooth loss separately, there were only associations in those with tooth loss. These findings suggest that SNPs could predispose individuals with tooth loss to have OPMD/OSCC. In this group, the allele A for the rs1533767 (WNT11) increased the risk for OPMD/OSCC two times (OR = 2.03, $p = 0.05$), while the allele C in AXIN2 (rs3923087 and rs11867417) had a protective effect for OPMD/OSCC (OR = 0.53, $p = 0.05$ and OR = 0.42, $p = 0.00$, respectively). However, after adjustments for sex, age, and smoking habits, only rs11867417 (AXIN2) remained statistically significantly associated with OPMD/OSCC and tooth loss.

Multiple corrections are necessary when testing many pairs of groups.[38] In our study, there were no significant associations after Bonferroni correction regarding the association of each SNP and the occurrence of the

TABLE 6 Full model association results from the variants and OPMD/OSSC in patients with and without tooth loss.

Gene (SNP)	Allele D/d	Model	Individuals with tooth loss				Individuals without tooth loss			
			Affected	Unaffected	*p*-value	OR	Affected	Unaffected	*p*-value	OR
GSK3B (rs9879992)	G/A	Genotypic	4/15/15	10/26/24	0.80		1/3/6	6/9/9	0.43	
		Allele	23/45	46/74	0.53	0.82	5/15	21/27	0.15	0.43
		Dominant	19/15	36/24	0.69	0.93	4/6	15/9	0.23	0.40
		Recessive	4/30	10/50	0.52	0.67	1/9	6/18	0.32	0.33
WNT11 (rs1533767)	A/G	Genotypic	2/12/16	3/13/43	0.08		0/3/7	1/4/19	0.57	
		Allele	18/46	19/99	**0.05**	**2.03**	3/17	6/42	0.78	1.23
		Dominant	16/16	16/43	**0.03**	**1.67**	3/7	5/19	0.56	1.63
		Recessive	2/30	3/56	0.81	1.24	0/10	1/23	0.51	
AXIN2 (rs11867417)	C/T	Genotypic	6/12/16	24/24/16	**0.04**		1/6/4	6/8/10	0.39	
		Allele	24/44	72/56	**0.00**	**0.42**	8/14	20/28	0.67	0.80
		Dominant	18/16	48/16	**0.03**	**0.37**	7/4	14/10	0.77	1.25
		Recessive	6/28	24/40	**0.04**	**0.36**	1/10	6/18	0.27	0.30
AXIN2 (rs3923087)	C/T	Genotypic	7/13/14	24/20/19	0.20		1/3/6	7/7/11	0.50	
		Allelic	27/41	68/58	**0.05**	**0.56**	5/15	21/29	0.26	0.46
		Dominant	20/14	44/19	0.27	0.62	4/6	14/11	0.39	0.52
		Recessive	7/27	24/39	0.08	0.42	1/9	7/18	0.25	0.27
AXIN2 (rs2240308)	C/T	Genotypic	5/11/19	11/18/38	0.87		0/5/6	5/5/15	0.13	
		Allele	21/49	40/94	0.98	1.01	5/17	15/35	0.52	0.68
		Dominant	16/19	29/38	0.81	1.10	5/6	10/15	0.76	1.25
		Recessive	5/30	11/56	0.78	0.85	0/11	5/20	0.11	
ERN1 (196929)	C/T	Genotypic	12/9/13	16/25/19	0.33		1/5/5	3/9/11	0.91	
		Allele	33/35	57/63	0.89	1.04	7/15	15/31	0.95	0.96
		Dominant	21/13	41/19	0.51	0.75	6/5	12/11	0.89	1.10
		Recessive	12/22	16/44	0.37	1.50	1/10	3/20	0.74	0.66

Note: Bold indicates statistically significant *p*-values under the threshold 0.05. Individuals with tooth loss are those with 6 or more missing teeth. Individuals without tooth loss are those with 5 or less missing teeth (reference group). D is the minor allele and d is the common allele. Affected, individuals with OPMD/OSCC; Unaffected, individuals without OPMD/OSCC; OR, odds ratio.

outcome (OPMD/oral cancer) among those with and without tooth loss. It was found in the literature some similar results regarding the loss of association after Bonferroni correction in genetic studies.[27,39,40] One possible explanation is that the Bonferroni correction reduces statistical power, making it more challenging to detect true associations, especially in genetic studies, where the goal is often to identify subtle effects.[38]

The evidence regarding the relationship between tooth loss and oral cancer remains inconsistent.[15,16,18,41] Previous studies did not find a significant association between these conditions.[16,41] However, after adjustment for age, sex, smoking, alcohol use, body mass index, and history of diabetes mellitus, an inverse association was found in the group with 16 or more tooth loss (OR = 3.64, 95% CI = 1.15–11.53, $p = 0.03$).[16] On the other hand, a meta-analysis found that tooth loss was significantly associated with head and neck cancer risk (Relative Risk = 1.52, 95% CI = 1.14–1.90, $p < 0.001$), with a positive nonlinear correlation between them ($p = 0.001$ for nonlinearity).[15] When analyzing the association per tooth lost, no significant increase in risk was confirmed.[16] Moreover, no association was found between the loss of one to six teeth and head and neck cancer (OR = 1.89, 95%CI = 1.27–2.80, $p = 0.001$).[18] However, exposure to more teeth loss, more than 15 and more than 20, increased the risk of head and neck cancer by 1.72 (OR = 1.72, 95%CI = 1.26–2.36, $p = 0.001$) and 1.89 times (OR = 1.29, 95%CI = 0.52–3.20, $p = 0.59$), respectively,[18] consistent with our data where an increased risk was observed for edentulism. Considering several types of cancer, this association was confirmed (OR = 2.12, $p = 0.0006$, 95%CI = 1.37–3.38).[14,17,27]

TABLE 7 Logistic regression analysis of associations between the variants and OPMD/OSSC in patients with tooth loss.

Gene (SNP)	Allele D/d	Adjustment	Odds ratio	*p*-value
GSK3B (rs9879992)	G/A	Sex	0.83	0.55
		Ethnicity	0.83	0.56
		Smoking habits	0.85	0.59
WNT11 (rs1533767)	A/G	Sex	2.02	0.06
		Ethnicity	1.94	0.07
		Smoking habits	1.91	0.08
AXIN2 (rs11867417)	C/T	Sex	0.48	**0.01**
		Ethnicity	0.50	**0.01**
		Smoking habits	0.52	**0.02**
AXIN2 (rs3923087)	C/T	Sex	0.62	0.08
		Ethnicity	0.63	0.09
		Smoking habits	0.61	0.07
AXIN2 (rs2240308)	C/T	Sex	0.99	0.97
		Ethnicity	1.00	0.99
		Smoking habits	0.98	0.95
ERN1 (196929)	C/T	Sex	1.03	0.88
		Ethnicity	1.02	0.93
		Smoking habits	1.02	0.93

Note: Bold indicates statistically significant *p*-values under the threshold 0.05. D is the minor allele and d is the common allele.

The inconsistency in the association between tooth loss/edentulism and oral cancer in the literature might be explained by oral hygiene habits.[41] Toothbrushing is an effective approach to control oral bacteria and decrease the risk of dental caries and periodontal disease, and consequently, tooth loss. Improved oral hygiene habits adopted by the population over the years could have been helpful in controlling oral bacteria and decreasing the chance of carcinogenesis.[16] Additionally, as people improve their living standards and dietary patterns, the chances of OPMD/cancer could be modulated.[42] In this study, the included population is relatively old and grew up in a period when dental extractions were a more frequent dental practice in Brazil. This could contribute to bias in the number of missing teeth.

The SNPs tested here were previously associated with both oral cancer[24,25] and overall cancer,[26] as well as craniofacial phenotypes such as oral leukoplakia,[27,28] tooth loss,[27] tooth agenesis,[29,30] dental caries, periodontitis, and periapical lesions in previous studies.[14,17,31] One study found that rs11867416 in AXIN2 was associated with the loss of teeth/edentulism,[27] consistent with our data. The lack of statistical association between the other SNPs and OPMD/OSCC in the group with tooth loss could be related to differences in the genetic background between populations and the impact of environmental factors on genetic expression.

This study assessed the relationship between tooth loss/edentulism and OPMD/OSCC. The number of missing teeth for each patient was obtained through a dental examination conducted by a single dentist. The reason for losing teeth (dental extraction or agenesis) was accessed through patient interviews, which could introduce some limitations. However, since tooth loss is a significant and often unpleasant event, the likelihood of patients not remembering the reason for the loss is low,[43] reducing the chance of memory bias in our study. We acknowledge the limitation that unreported cases of tooth agenesis could exist, although this condition has also been associated with cancer by WNT genes.[29,30,44] Another limitation is our inability to investigate specific reasons for extraction (e.g., dental caries, periodontal diseases, endodontic reasons) due to the absence of an electronic database allowing the collection of previous dental information for these patients. Nonetheless, tooth loss has been considered a proxy for poor oral health and dental diseases, which may contribute to systemic inflammation, local aggressive immune responses, and cancer,[10,15,16] supporting the focus of our investigation. We were unable to assess the impact of oral hygiene habits and dietary patterns on the association between tooth loss and OPMD/OSCC. Additionally, the sample size of this study may not be large enough to detect statistically significant differences in the other SNPs tested

among those with and without tooth loss. Future studies investigating this relationship should consider a larger population and include oral hygiene habits and dietary patterns as potential covariates in genetic and epidemiological analyses. Furthermore, studies focusing on the potential effects of oral bacteria and associated biological mechanisms on OSCC remain necessary.

To our knowledge, this is the first study conducted to investigate the association between tooth loss and OPMD/OSCC in Brazil, using both epidemiological and genetic data. We found only one previous multicentric case–control study conducted in Latin America regarding oral health and hygiene in relation to head and neck cancer.[2] However, this study did not include any city in the Northeast of Brazil, nor did it directly assess the relationship studied here. Our results strengthen the link between oral health and premalignant/malignant oral disorders, justifying the importance of clinically monitoring patients at high risk for tooth loss related to dental conditions to decrease the risk for OPMD/OSCC. Our findings support the implementation of preventive and diagnostic measures that could positively impact cancer outcomes and survival rates.

5 | CONCLUSION

This study leads to the conclusion that tooth loss could increase the risk for oral potentially malignant disorders and oral squamous cell carcinoma. Edentulism increased the risk for oral potentially malignant disorders and oral squamous cell carcinoma, even after individual adjustments for educational level and smoking habits. However, when this association was adjusted for both covariates simultaneously, the result lost its significance. Genetic analyses show a significant association between oral potentially malignant disorders and oral squamous cell carcinoma and rs11867417 (AXIN2) in those with tooth loss, strengthening the link between oral health and premalignant/malignant oral disorders.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. World Health Organization. Oral health; 2023. https://www.who.int/news-room/fact-sheets/detail/oral-health#:~:text=Edentulism%20(total%20tooth%20loss)&text=The%20estimated%20global%20average%20prevalence,aged%2020%20years%20or%20older. Cited June 27, 2023
2. Guha N, Boffetta P, Wünsch Filho V, et al. Oral health and risk of squamous cell carcinoma of the head and neck and esophagus: results of two multicentric case-control studies. *Am J Epidemiol*. 2007;166(10):1159-1173.
3. Passarelli PC, Pagnoni S, Piccirillo GB, et al. Reasons for tooth extractions and related risk factors in adult patients: a cohort study. *Int J Environ Res Public Health*. 2020;17(7):2575.
4. Upadhyaya C, Humagain M. The pattern of tooth loss due to dental caries and periodontal disease among patients attending dental department (OPD), Dhulikhel Hospital, Kathmandu University Teaching Hospital (KUTH). *Nepal Kathmandu Univ Med J*. 2009;7(25):59-62.
5. Pitts NB, Zero DT, Marsh PD, et al. Dental caries. *Mol Med Microbiol Second Ed*. 2017;3:945-955.
6. Curtis MA, Diaz PI, Van Dyke TE. The role of the microbiota in periodontal disease. *Periodontology 2000*. 2020;83:14-25.
7. Tuominen H, Rautava J. Oral microbiota and cancer development. *Pathobiology*. 2021;88:116-126.
8. Chattopadhyay I, Verma M, Panda M. Role of oral microbiome signatures in diagnosis and prognosis of oral cancer. *Technology in Cancer Research and Treatment*. Vol 18. SAGE; 2019.
9. Hoare A, Soto C, Rojas-Celis V, Bravo D. Chronic inflammation as a link between periodontitis and carcinogenesis. *Mediators of Inflammation*. Hindawi Limited; 2019.
10. Michaud DS, Fu Z, Shi J, Chung M. Periodontal disease, tooth loss, and cancer risk. *Epidemiol Rev*. 2017;39(1):49-58.
11. Michaud DS, Lu J, Peacock-Villada AY, et al. Periodontal disease assessed using clinical dental measurements and cancer risk in the ARIC study. *J Natl Cancer Inst*. 2018;110(8):843-854.
12. Corbella S, Veronesi P, Galimberti V, Weinstein R, Del FM, Francetti L. Is periodontitis a risk indicator for cancer? A meta-analysis. *PloS One*. 2018;13(4):e0195683.
13. The Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. *Nature*. 2013;486(7402):207-214.
14. Bezamat M, Rothenberger S, Vieira AR. Genetic contribution to cancer risk in patients with tooth loss: a genetic association study. *Sci Rep*. 2022;12(1):1-7. doi:10.1038/s41598-022-20556-2
15. Shi J, Leng W, Zhao L, et al. Tooth loss and cancer risk: a dose-response meta analysis of prospective cohort studies. *Oncotarget*. 2018;9(19):15090-15100.
16. Zuo C, Zhu Y, Wang X, Zeng X, Huang C. Tooth loss and risk of oral squamous cell carcinoma in Chinese Han population. *Int J Clin Exp Med*. 2015;8(11):21893-21897.
17. Lucas MBC. Oral Health Outcomes As Potential Indicators of Cancer Experience. Dissertion. University of Pittsburgh; 2021.
18. Zeng XT, Luo W, Huang W, Wang Q, Guo Y, Leng WD. Tooth loss and head and neck cancer: a meta-analysis of observational studies. *PloS One*. 2013;8(11):e79074.
19. Vieira AR, Hilands KM, Braun TW. Saving more teeth—a case for personalized care. *J Pers Med*. 2015;5(1):30-35.
20. Vandenbroucke JP, Elm Von E, Altman D, et al. Strengthening the reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. *Int J Surg*. 2014;12:1500-1524.

21. Little J, Higgins JPT, Ioannidis JPA, et al. STrengthening the REporting of genetic association studies (STREGA)—an extension of the strobe statement. *PLoS Med*. 2009;6(2):151-163.
22. Tezal M, Scannapieco FA, Wactawski-Wende J, et al. Dental caries and head and neck cancers. *JAMA Otolaryngol Head Neck Surg*. 2013;139(10):1054-1060.
23. Ranade K, Chang MS, Ting CT, et al. High-throughput genotyping with single nucleotide polymorphisms. *Genome Res*. 2001;11(7):1262-1268.
24. Andrade Filho PA, Letra A, Cramer A, et al. Insights from studies with oral cleft genes suggest associations between WNT-pathway genes and risk of oral cancer. *J Dent Res*. 2011;90(6):740-746.
25. De FEM, Machado RA, De SE, et al. Polymorphisms associated with oral clefts as potential susceptibility markers for oral and breast cancer. *Arch Oral Biol*. 2019;99:9-14. doi:10.1016/j.archoralbio.2018.12.004
26. Li X, Li Y, Liu G, Wu W. New insights of the correlation between AXIN2 polymorphism and cancer risk and susceptibility: evidence from 72 studies. *BMC Cancer*. 2021;21(1):1-15.
27. Bezamat M, Harrison B, Zhou Y, et al. Phenome-wide scan finds potential orofacial risk markers for cancer. *Sci Rep*. 2020;10(1):1-10. doi:10.1038/s41598-020-61654-3
28. Zhang X, Kim KY, Zheng Z, Kim HS, Cha IH, Yook JI. Snail and Axin2 expression predict the malignant transformation of oral leukoplakia. *Oral Oncol*. 2017;73:48-55. doi:10.1016/j.oraloncology.2017.08.004
29. Letra A, Menezes R, Granjeiro JM, Vieira AR. AXIN2 and CDH1 polymorphisms, tooth agenesis, and oral clefts. *Birth Defects Res A Clin Mol Teratol*. 2009;85(2):169-173.
30. Cardoso E, Artelli DRB, Machado RA, et al. Nonsyndromic cleft lip and palate, gastric cancer and tooth agenesis. *Med Oral Patol Oral Cir Bucal*. 2018;1(23):e44-e48.
31. Bezamat M, Deeley K, Khaliq S, et al. Are mTOR and ER stress pathway genes associated with oral and bone diseases? *HHS Public Access*. 2020;53(3):235-241.
32. Liu F, Millar SE. Wnt/β-catenin signaling in oral tissue development and disease. *J Dent Res*. 2010;89(4):318-330.
33. Nader NHR, Gataa IS, Mohammad DN. CD34 and Wnt3 expression in potentially malignant oral disorders. *J Baghdad Coll Dent*. 2017;29(3):59-67.
34. Zhan T, Rindtorff N, Boutros M. Wnt signaling in cancer. *Oncogene*. 2017;36(11):1461-1473. doi:10.1038/onc.2016.304
35. Ejaz I, Ghafoor S. Wnt signaling pathway in oral lesions. *J Pak Med Assoc*. 2019;69(11):1687-1692.
36. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet*. 2007;81(3):559-575.
37. Brunotto M, Zarate AM, Bono A, Barra JL, Berra S. Risk genes in head and neck cancer: a systematic review and meta-analysis of last 5 years. *Oral Oncol*. 2014;50:178-188.
38. Noble WS. How does multiple testing correction work? *Nat Biotechnol*. 2009;27(12):1135-1137.
39. Machado RA, de Oliveira Silva C, Persuhn DC, et al. Interactions between superoxide dismutase and paraoxonase polymorphic variants in nonsyndromic cleft lip with or without cleft palate in the Brazilian population. *Environ Mol Mutagen*. 2019;60(2):185-196.
40. Vijayan V, Ummer R, Weber R, Silva R, Letra A. Association of WNT pathway genes with nonsyndromic cleft lip with or without cleft palate. *Cleft Palate-Craniofacial J*. 2018;55(3):335-341.
41. Tsukamoto M, Naito M, Wakai K, et al. Tooth brushing, tooth loss, and risk of upper aerodigestive tract cancer: a cohort study of Japanese dentisits. *Nagoya J Med Sci*. 2021;83(2):331-341.
42. Rodríguez-Molinero J, Migueláñez-Medrán BDC, Puente-Gutiérrez C, et al. Association between oral cancer and diet: an update. *Nutrients*. 2021;13:1299.
43. Gerritsen AE, Allen PF, Witter DJ, Bronkhorst EM, Creugers NHJ. Tooth loss and oral health-related quality of life: a systematic review and meta-analysis. *Health Qual Life Outcomes*. 2010;5:8.
44. Küchler EC, Lips A, Tannure PN, et al. Tooth agenesis association with self-reported family history of cancer. *J Dent Res*. 2013;92(2):149-155.

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Risk of orofacial clefts in relation to maternal body mass index, diabetes and hypertension

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Abstract

Background: To investigate if maternal body mass index, diabetes and hypertension are associated with orofacial clefts.

Methods: Case-control study. Information from 53,188 live births with and without orofacial cleft registered at USA Vital Statistics Natality Birth Data between 2017 and 2021. Case group consisted of all affected live births diagnosed with orofacial clefts (13,297 cases). Comparison group consisted of 39,891 live births without clefts or any other congenital malformation. Information about orofacial cleft cases were compared with the comparison group. The unadjusted and adjusted Odds Ratios were estimated to evaluate the strength of association between mother's pre-pregnancy body mass index, maternal diabetes and hypertension and orofacial cleft occurrence, assuming a p value < 0.05 and 95% confidence intervals (95% C.I.) for statistically significant differences.

Results: Mother's pre-pregnancy body mass index, maternal diabetes and maternal hypertension are conditions associated with an increased risk of orofacial cleft development in the child (OR = 1.08, p = 0.004, 95% C.I. = 1.024-1.149; OR = 1.32, 95%, p = 0.000, 95% C.I. = 1.202-1.444; and OR = 1.35, p = 0.000, 95% C.I. = 1.239-1.484; respectively). Maternal ethnicity, sex of infant, and cigarette smoking pregnancy remained as covariates after adjustments in all logistic regression models.

Conclusion: Due to the increased prevalence of obesity, diabetes, and hypertension, and also to their association with congenital malformations, such as clefts, it is recommended that mothers planning to become pregnant to follow healthy habits, maintain healthy weight, and be screened for possible diabetes or hypertension prior to conception and early in pregnancy.

Keywords: Body mass index; cleft lip; cleft palate; diabetes complications; hypertension.

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1 Introduction

Orofacial clefts (OC) are the most common congenital craniofacial anomalies with a prevalence rate of approximately 1 in 700 live births worldwide worldwide [1]. Clefts are generally classified as cleft lip with or without palate (CL+P) and cleft palate only (CP) [2]. OC have a complex etiology in which genetic and environmental factors act together, or in isolation in some instances, causing alterations in the fetus embryologic process [3].

More than one gene are related to this malformation, and since each of these individual genes has a small contribution, it is difficult to detect them, because affected individuals can express their conditions due to a variety of different mechanisms that may involve different pathways. The environment can modify these genes and predisposition to the occurrence of clefts is difficult to study [3,4]. Defining the role of potential environmental factors could reduce the prevalence of this congenital malformation [5].

Several environmental factors have been suggested to be associated with OC, such as smoking tobacco, drinking alcohol, or occupational exposures [6]. Also, studies demonstrated that the risk for birth defects increases with increasing of maternal body mass index (BMI), diabetes and hypertension [7–9]. An intrauterine hyperglycemic environment may cause oxidative stress and increase the risk of congenital anomalies in developing fetuses[10]. The pregestational diabetes [11] and gestational diagnosis of diabetes mellitus indicate possible factors that lead to the OC [12]. Hypertension showed to increase risk of major non-chromosomal congenital malformations in all mothers with this condition [13]. Both maternal obesity [14], and underweight[15] have been found to be associated with clefts. Maternal weight gain seems to increase the risk for diabetes and hypertension [7], and these conditions could act synergistically in the pathogenesis of craniofacial abnormalities [16].

Obesity, diabetes and hypertension are significant public health problems [17], and their rising rates in the population may result in an increased burden of birth defects, such as clefts [7]. However, issues regarding the relationship between these diseases and OC are still insufficiently studied [7]. Authors highlight the importance of understand the aspects related to OC etiology in order to prevent them when possible [6]. Therefore, the objective of this study was to investigate if maternal body mass index, diabetes and hypertension are associated with OC.

2 Methods

This is a case-control study conducted with data from 53,188 live births collected from the USA Vital Statistics Natality Birth Data for the last five years available (2017 to 2021). Natality data show demographic and health data for births occurring in the United States for each year. These data are provided to the National Center for Health Statistics (NCHS) through the National Vital Statistics System (NVSS), and are based on the Standard Certificate of Live Birth, which was fully implemented in all States of the United States (USA) and District of Columbia in 2016. The vital statistics data are interpreted according to various qualifying factors and methods of classification in order to verify their value for utilization in most general purposes [18], making these data the most comprehensive and reliable USA national source of birth information [19].

This study used public-use natality data from an open access information system, in which the data are presented without identifying the subjects. Investigators here had no contact with human subjects. Also, this study was reported according to the recommended STROBE (STrengthening the Reporting of Observational studies in Epidemiology) statement [20].

The cases consisted of all affected live births diagnosed with OC registered in the NVSS (13,297 cases in total, being 9,596 with CL+P, and 3,701 with CP) from 2017 to 2021. OC identified at birth were reported on the revised 2003 birth certificate, having a checkbox ("yes", "no", "unknown or not stated") for each type (CL+P and CP). It was only considered as cases those with a confirmed register (Yes) of presence of CL+P and/or CP, with or without any other congenital malformation (syndromic and non-syndromic OC, respectively). Infants whose birth certificate did not indicate the presence of OC ("unknown or not stated") were excluded for the study. Cases were combined in a 1:3 ratio with a comparison group of 39,891 live births without history of clefts or any other congenital malformation that were born during the same period. The selection of the control group was done randomly after remove from the population all births considered as cases, births without register for OC, and births that have a positive or unknown diagnosis for any of other 11 congenital malformations (cyanotic congenital heart disease, hypospadias, Down syndrome, gastroschisis, suspected chromosomal disorder, meningomyelocele/spina bifida, congenital diaphragmatic hernia, limb reduction defect, anencephaly, and omphalocele) without OC combined. For Down syndrome and suspected chromosomal disorder, it was also excluded those with "pending" confirmation (Figure 1).

Table 1. Unadjusted and adjusted Odds Ratios (OR) between independent variables and orofacial clefts

	Orofacial clefts				Cleft Lip/Palate				Cleft Palate Only			
	Crude OR	p-value	Adjusted OR	p-value	Crude OR	p-value	Adjusted OR	p-value	Crude OR	p-value	Adjusted OR	p-value
Mother's pre-pregnancy BMI	1.14	**0.000**	1.08	**0.004**	1.12	**0.000**	1.08	**0.02**	1.13	**0.000**	1.06	0.21
Maternal pre pregnancy diabetes	2.57	**0.000**	2.17	**0.000**	2.25	**0.000**	1.72	**0.000**	2.00	**0.000**	2.24	**0.000**
Maternal gestational diabetes	1.20	**0.000**	1.17	**0.002**	1.33	**0.002**	1.10	0.085	1.25	**0.000**	1.26	**0.005**
Maternal diabetes	1.38	**0.000**	1.32	**0.000**	1.29	**0.000**	1.21	**0.000**	1.37	**0.000**	1.44	**0.000**
Maternal pre hypertension	1.49	**0.000**	1.34	**0.001**	1.32	**0.000**	1.35	**0.001**	1.59	**0.000**	1.16	0.291
Maternal gestational hypertension	1.34	**0.000**	1.33	**0.000**	1.31	**0.000**	1.23	**0.000**	1.22	**0.000**	1.39	**0.000**
Maternal hypertension	1.40	**0.000**	1.35	**0.000**	1.34	**0.000**	1.28	**0.000**	1.33	**0.000**	1.36	**0.000**

Bold indicates statistically significant p-values under the threshold 0.05

Mother's pre-pregnancy Body Mass Index (BMI) indicated mother's body fat based on her height and pre-pregnancy weight [18]. It was calculated as: [mother's pre-pregnancy weight (lb) / [mother's height (in)]2] x 703. The categories for BMI classification adopted in the vital record were established by the National Health, Lung and Blood Institute [21] as underweight (BMI <18.5), normal weight (BMI 18.5–24.9), overweight (BMI 25–29.9), class I obese (BMI 30–34.9), class II obese (BMI 35–39.9), and class III obese (BMI >40).

Information about the presence or absence of maternal diabetes and maternal hypertension are available in the USA Vital Statistics Natality Birth Data for both pre pregnancy and gestational period, identified as "yes" and "no". These data are collected directly from the Facility Worksheet for a Live Birth Certificate, which is typically completed by a medical professional based on mother's prenatal care records. The dataset does not inform if these diseases were controlled or uncontrolled [18].

Maternal diabetes mellitus was defined as having type 1 or type 2 diabetes diagnosed prior to the pregnancy, as pre gestational diabetes, or during the pregnancy, as gestational diabetes [10]. Maternal hypertension was considered as the elevation of blood pressure above normal for age, gender, and physiological condition diagnosed prior to the onset of the pregnancy (pre gestational hypertension) or during this pregnancy (gestational hypertension). This elevation of blood pressure should be >140mmHg (systolic blood pressure) > 90mmHg (diastolic blood pressure) on two separate occasions at least four hours apart after 20 weeks of pregnancy when previous blood pressure was normal [22]. This study also analyzed maternal diabetes and maternal hypertension without distinguishing between gestational and prepregnancy period in order to minimize chances of bias due to misclassification.

Information regarding maternal age, maternal ethnicity, sex of infant, birth order, history of abortion, interval since the last pregnancy, number of prenatal care visits, month of prenatal care began, cigarettes smoked before or during pregnancy, previous preterm birth, infertility treatment, previous cesarean delivery, infections during pregnancy, and plurality were collected and used as covariates.

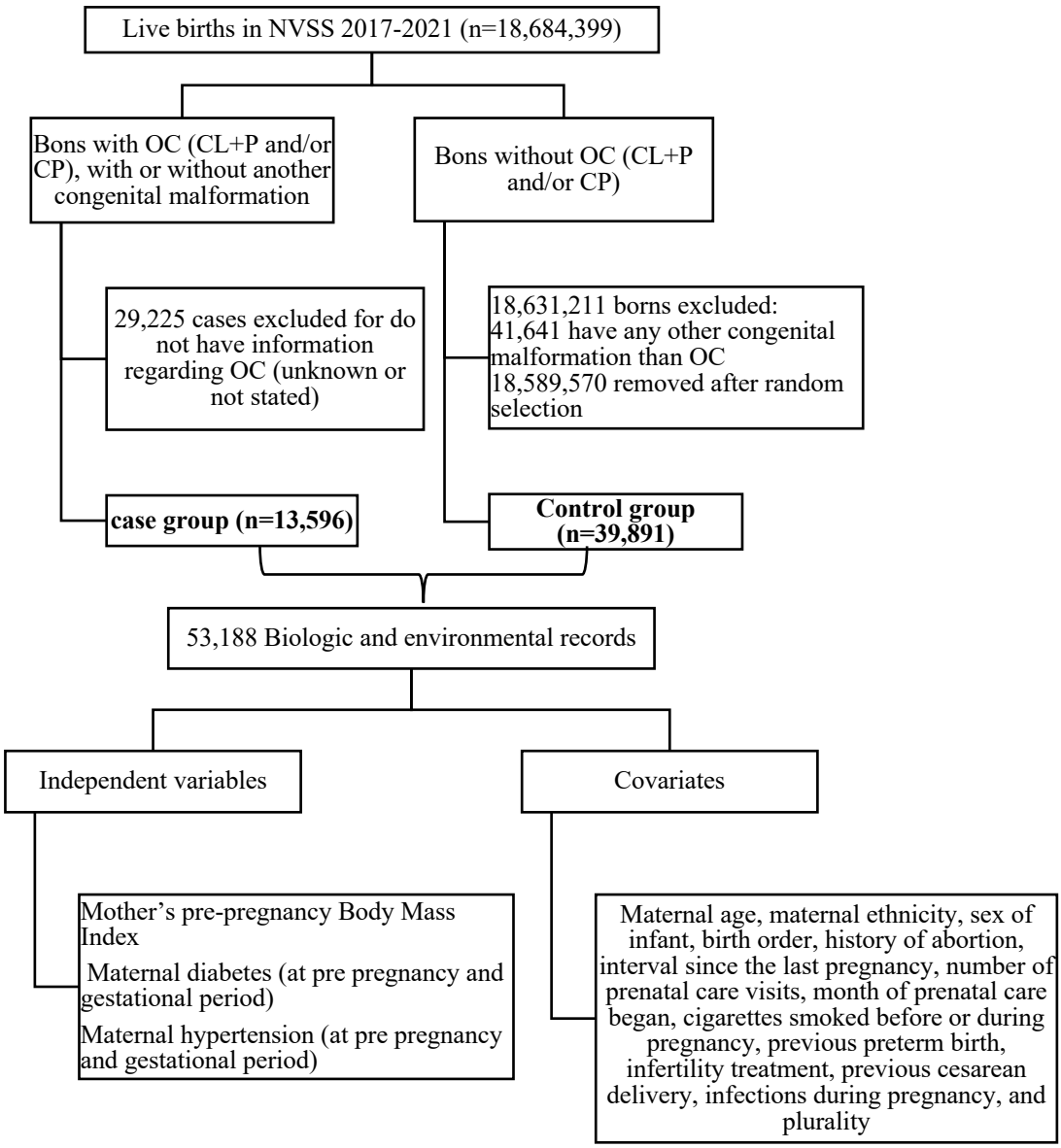
All variables were analyzed previously according to the criteria of incompleteness proposed by Romero and Cunha [23,24] as excellent (< 5%), good (5% to 9.9%), regular (10% to 19.9%), poor (20% to 49.9%), and very poor (≥ 50%). Differences between cases and controls were evaluated using chi-square. In addition, analyses were done by cleft type (cleft palate only and cleft lip with/without cleft palate) to explore the possibility of different etiological mechanisms that may affect differently the two cleft types [25]. Unadjusted Odds Ratios (OR) were estimated to evaluate the strength of association between mother's pre-pregnancy BMI, maternal diabetes and hypertension, and types of OC occurrence. Because overweight and obesity are risk factors for both gestational diabetes and hypertensive conditions19, stratified analyses were done according to the mother's pre-pregnancy BMI status (normal x underweight/obese/overweight). Potential associations between the covariates and clefts were also determined. Then, logistic regression models were used to assess the adjusted OR, adjusting by those covariates that have been shown statistically significant differences associated with clefts. All these analyzes were done using STATA (Software for Statistics and Data Science), assuming a p-value ≤ 0.05 and 95% confidence intervals (95% C.I.) not involving 1.0 as statically significant.

3 Results

From 53,188 live births collected from the USA Vital Statistics Natality Birth Data for the last five years available (2017 to 2021), 13,297 had OC, 31,074 (58.42%) mothers had altered BMI (underweight, overweight or obesity), 690 (1.30%) had pre gestational diabetes, 4,014 (7.55%) mothers had gestational diabetes, 1,302 (2.45%) mothers had pre hypertension, and 4,318 (8.12%) mothers had gestational hypertension. All variables included in this study had an excellent to good degree of incompleteness, with missing data ranging between 0% to 3.03%, with the exception of "interval since the last pregnancy" that had a poor degree of completeness, missing 43% of data. Comparing the groups, only maternal age did not show to have a statistically significant difference (p=0.959) between cases and controls.

Stratified analyses showed that the risk for OC in the child was lower among mothers with diabetes or hypertension who had normal BMI (OR=1.31 and OR=1.35 respectively), comparing with those underweight, obese or overweight (OR=1.35 and OR=1.37, respectively). However, none of these analyses were statistically significant (p=0.653, 95%C.I= 1.138-1.508 and p=0.891, 95%C.I. = 1.191-1.540 respectively). The unadjusted and adjusted OR regarding the strength of associations between mother's pre-pregnancy BMI, maternal diabetes and hypertension and OC occurrence can be found in Table 1. Maternal ethnicity, sex of infant, cigarettes smoked before or during pregnancy, remained as covariates after adjustments in all logistic regression models.

Figure 1. Overall study design



4 Discussion

The findings of this study demonstrated that mother's pre-pregnancy BMI, maternal diabetes and maternal hypertension are conditions associated with an increased risk of OC development in the child. These results reinforce the need to follow up pregnant women in order to control the identified risk factors.

Mother's pre-pregnancy BMI was associated with OC, confirming findings that underweight or overweight/obese women are at higher risk to have a child with clefts, compared to women of recommended weight [7,14,26]. The underlying mechanisms for the increased risk of OC in both underweight and obese mothers are unclear and could be very different [25]. However, several mechanisms have been hypothesized as potential explanations for this association, such as insulin resistance and hyperglycemia during embryogenesis (also common for diabetes) [16]. Maternal obesity [16,25,27] and maternal underweight [25,28] may also be associated with poor nutrient intake and low blood levels of nutrients critical for fetal development, resulting in poor reproductive outcomes. Some population studies using vital records of some United States regions have shown positive associations between maternal obesity and risk of orofacial clefts [9,29]. A study using Western Australian Birth Defects Registry data found a twofold-increased odds of having an infant with OC among women with pre-pregnancy obesity [30]. Analyses of six large case-control studies of OC from Northern Europe and the USA also found an increased risk of clefts in obese mothers compared with normal-weight mothers and suggested that extremes of weight may have a specific effect on palatal development [25]. Given the increased risk for OC and other birth defects associated with maternal obesity [16], preconception counseling should emphasize the importance of maintaining normal weight [9,14,29–31].

Pre-gestational and gestational diabetes are commonly linked to OC [32,33]. In this study, the presence of maternal diabetes was associated with an increased risk for clefts, with a twofold increased odds for clefts among mothers who had pre pregnancy diabetes, confirming results found in other studies [10,32,34]. Although the mechanisms underlying associations between diabetes mellitus and birth defects are not completely understood, it seems that hyperglycemia during embryogenesis could increase oxidative stress, epigenetic changes, hypoxia, and apoptosis, contributing to DNA damage and a higher risk for congenital malformations, such as clefts [12,33,35,36].

Maternal hypertension is another pregnancy complication that can potentially increase the risk for clefts [7,37]. Findings from the present study confirmed the association between

pre or gestational maternal hypertension and OC in the offspring. Although the stratified analysis did not show statistically significant increases in the risk for obese hypertensive mothers, the literature revealed that the risk for OC seems to be elevated among mothers with hypertension who were also obese [7]. Due to the increased prevalence of obesity, diabetes, and hypertension, and also to their association with congenital malformations, such as OC, it is recommended that mothers planning to become pregnant to follow healthy habits, maintain healthy weight, and be screened for possible diabetes or hypertension prior to conception and early in pregnancy [7].

As strengths of this study, it has a full coverage of a nationwide population of mother-infant pairs, a large sample size, and low levels of missing data, which allow to better examine rare conditions, such as OC. Also, the analyzes done here took into account the adjustment for several covariates to confirm the findings. Here we opted to develop a case-control study because even though OC are among one of the most common congenital malformations of the craniofacial region [38,39], they were also a rare event considering all population, which is one of the indications of using this kind of study design. Consequently, the association analyzes were done by OR, and the use of logistic regression becomes the better approach to obtain the adjusted OR when there is a dichotomous outcome [40]. Other studies were done using this kind of study design to assess the association between some risk factors and birth congenital anomalies [41,42].

This study has some limitations inherent to the design of the case-control study, such as the convenience sample and the retrospective measurement of the predictive variables (susceptible to memory bias) [40]. Belong that, the principal limitation here would be the use of vital statistics data, which is susceptible to bias in the original records or in tabulation due to missing or misclassifications. However, this limitation does not decrease the value of USA Vital Statistics Natality Birth Data since they are analyzed by various qualifying factors in order to increase its reliability [18]. These data are the only USA database for births that is national in scope, and our analyzes revealed an excellent to good degree of information incompleteness, strengthening their use as a comprehensive and reliable USA national source of birth data. Another limitation would be the identification of OC cases solely from birth certificates registry due to chances of imprecision in cleft registration. However, OC are a type of congenital anomaly likely to be observable at birth, with relatively well-defined categories. Also, the registration of the presence or absence of OC in the birth certificate is done after medical evaluation. These factors minimize chances of OC misclassification (i.e., the infants who did not have diagnosis of OC at birth but were diagnosed later in life were treated as not

having OC in this study) and increase the sensitivity. Furthermore, several studies have been performed using this approach to select cases and assess the association between some risk factors and birth congenital anomalies and in USA [9,29,43–45] and in other countries [46–48].

5 Conclusion

The findings of this study demonstrated that mother's pre-pregnancy BMI, maternal diabetes and maternal hypertension are conditions associated with an increased risk of OC development in the child. These results, along with the increased prevalence of obesity, diabetes, and hypertension, in the population, reinforce that mothers planning to become pregnant should follow healthy habits, maintain healthy weight, and be screened for possible diabetes or hypertension prior to conception and early in pregnancy.

References

1. Mossey PA, Little J, Munger RG, Dixon MJ, Shaw WC. Cleft lip and palate. The Lancet [Internet]. 2009;374(9703):1773–85. Available from: http://dx.doi.org/10.1016/S0140-6736(09)60695-4
2. Nasreddine G, Hajj J El, Ghassibe-sabbagh M. Orofacial clefts embryology, classification, epidemiology, and genetics. Mutat Res [Internet]. 2021;787:108373. Available from: https://doi.org/10.1016/j.mrrev.2021.108373
3. Vieira AR. Basis of Inheritance in Humans. Monogr Oral Sci. 2022;30:1–19.
4. Vieira AR. Genetic basis of oral health conditions. Genetic Basis of Oral Health Conditions. 2021. 1–104 p.
5. Oginni FO, Adenekan AT. Prevention of orofacial clefts in developing world. Ann Maxillofac Surg. 2012;2(2):163.
6. Garland MA, Reynolds K, Zhou CJ. Environmental mechanisms of orofacial clefts. Birth Defects Res. 2020;1–39.
7. Kutbi HA. The Role of Obesity, Diabetes, and Hypertension in Cleft lip and Cleft Palate Birth Defects. Utah State University; 2014.
8. Gabbay-Benziv R. Birth defects in pregestational diabetes: Defect range, glycemic threshold and pathogenesis. World J Diabetes. 2015;6(3):481.

9. Marengo L, Farag NH, Canfield M. Body mass index and birth defects: Texas, 2005-2008. Matern Child Health J. 2013;17(10):1898–907.
10. Wu Y, Liu B, Sun Y, Du Y, Santillan MK, Santillan DA, et al. Association of maternal prepregnancy diabetes and gestational diabetes mellitus with congenital anomalies of the newborn. Diabetes Care. 2020 Dec 1;43(12):2983–90.
11. Temple R, Aldridge V, Greenwood R, Heyburn P, Sampson M, Stanley K. Association between outcome of pregnancy and glycaemic control in early pregnancy in type 1 diabetes: population based study. BMJ. 2002;325.
12. Kozma A. Gestational Diabetes Mellitus and the Development of Cleft Lip / Palate in Newborns. Acta Endocrinol (Copenh). 2019;15(1):118–22.
13. Li DK, Yang C, Andrade S, Tavares V, Ferber JR. Maternal exposure to angiotensin converting enzyme inhibitors in the first trimester and risk of malformations in offspring: A retrospective cohort study. BMJ (Online). 2011 Oct 29;343(7829).
14. Rankin J, Tennant PWG, Stothard KJ, Bythell M, Summerbell CD, Bell R. Maternal body mass index and congenital anomaly risk: A cohort study. Int J Obes [Internet]. 2010;34(9):1371–80. Available from: http://dx.doi.org/10.1038/ijo.2010.66
15. Bitsko RH, Reefhuis J, Louik C, Werler M, Feldkamp ML, Waller DK, et al. Periconceptional use of weight loss products including ephedra and the association with birth defects. Birth Defects Res A Clin Mol Teratol. 2008 Aug;82(8):553–62.
16. Stothard KJ, Tennant PWG, Bell R, Rankin J. Maternal Overweight and Obesity and the Risk of Congenital Anomalies A Systematic Review and Meta-analysis [Internet]. Vol. 301, JAMA. 2009. Available from: www.jama.com
17. Centers for Disease Control and Prevention. Health Topics [Internet]. [cited 2023 Apr 25]. Available from: https://www.cdc.gov/health-topics.html#cdc-atozlist
18. Center for Disease Control - CDC. User Guide to the 2021 Natality Public Use File.
19. Grünebaum A, McCullough LB, Orosz B, Chervenak FA. Neonatal mortality in the United States is related to location of birth (hospital versus home) rather than the type of birth attendant. Am J Obstet Gynecol. 2020 Aug 1;223(2):254.e1-254.e8.
20. Vandenbroucke JP von EEADGPMCPSPCSJ and EM. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. Vol. 12, Int J Surg 2014; 12: 1500-1524. 2020. p. 1500–24.
21. National Heart L and BI. Clinical Guidelines on the identification, evaluation and treatment of overweight and obesity in adults. Vol. 158, Archives of Internal Medicine. Washington DC: National Institutes of Health (NIH); 1998. 1855–1867 p.

22. American College of Obstetricians and Gynecologists' Committee on Practice Bulletins—Obstetrics. ACOG Practice Bulletin No. 203: Chronic Hypertension in Pregnancy. Obstet Gynecol [Internet]. 2019;133(1):e26–50. Available from: http://journals.lww.com/greenjournal
23. Romero DE, Cunha CB da. Avaliação da qualidade das variáveis epidemiológicas e demográfi cas do Sistema de Informações sobre Nascidos Vivos , 2002. Cad Saúde Públic. 2007;23(3):701–14.
24. Maia LT de S, Souza WV de, Mendes A da CG, Silva AGS da. Use of linkage to improve the completeness of the SIM and SINASC in the Brazilian capitals. Rev saúde pública [Internet]. 2017;51:112. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0034-89102017000100300
25. Kutbi H, Wehby GL, Uribe LMM, Romitti PA, Carmichael SL, Shaw GM, et al. Maternal underweight and obesity and risk of orofacial clefts in a large international consortium of population-based studies. Int J Epidemiol. 2017;46(1):190–9.
26. Blanco R, Colombo A, Suazo J. Maternal obesity is a risk factor for orofacial clefts: A meta-analysis. British Journal of Oral and Maxillofacial Surgery [Internet]. 2015;53(8):699–704. Available from: http://dx.doi.org/10.1016/j.bjoms.2015.05.017
27. King JC. Maternal obesity, metabolism, and pregnancy outcomes. Vol. 26, Annual review of nutrition. 2006. p. 271–91.
28. Hoellen F, Hornemann A, Haertel C, Reh A, Rody A, Schneider S, et al. Does Maternal Underweight Prior to Conception Influence Pregnancy Risks and Outcome? In Vivo (Brooklyn). 2014;28:1165–70.
29. Block SR, Watkins SM, Salemi JL, Rutkowski R, Tanner JP, Correia JA, et al. Maternal pre-pregnancy body mass index and risk of selected birth defects: Evidence of a dose-response relationship. Paediatr Perinat Epidemiol. 2013;27(6):521–31.
30. Oddy WH, De Klerk NH, Miller M, Payne J, Bower C. Association of maternal pre-pregnancy weight with birth defects: Evidence from a case-control study in Western Australia. Australian and New Zealand Journal of Obstetrics and Gynaecology. 2009;49(1):11–5.
31. Sato Y, Yoshioka E, Saijo Y, Miyamoto T, Sengoku K, Azuma H, et al. Population attributable fractions of modifiable risk factors for nonsyndromic orofacial clefts: A prospective cohort study from the Japan environment and children's study. J Epidemiol. 2021;31(4):272–9.

32. Correa A, Gilboa SM, Besser LM, Botto LD, Moore CA, Hobbs CA, et al. Diabetes mellitus and birth defects. Am J Obstet Gynecol. 2008;199(3):237.e1-237.e9.
33. Ornoy A, Reece EA, Pavlinkova G, Kappen C, Miller RK. Effect of maternal diabetes on the embryo, fetus, and children: Congenital anomalies, genetic and epigenetic changes and developmental outcomes. Birth Defects Res C Embryo Today. 2015 Mar 1;105(1):53–72.
34. Trindade-Suedam IK, Kostrisch LM von, Pimenta LAF, Negrato CA, Franzolin SB, Trindade Junior AS. Diabetes mellitus and drug abuse during pregnancy and the risk for orofacial clefts and related abnormalities. Rev Lat Am Enfermagem. 2016;24(0).
35. Eriksson UJ, Cederberg J, Wentzel P. Congenital malformations in offspring of diabetic mothers - Animal and human studies. Rev Endocr Metab Disord. 2003;4(1):79–93.
36. Gabbay-Benziv R. Birth defects in pregestational diabetes: Defect range, glycemic threshold and pathogenesis. World J Diabetes. 2015;6(3):481.
37. Cheshmi B, Jafari Z, Naseri MA, Davari HA. Assessment of the correlation between various risk factors and orofacial cleft disorder spectrum: a retrospective case-control study. Maxillofac Plast Reconstr Surg. 2020;42(1).
38. Mai CT, Isenburg JL, Canfield MA, Meyer RE, Correa A, Alverson CJ, et al. National population-based estimates for major birth defects, 2010–2014. HHS Public Access. 2019;111(18):1420–35.
39. Parker SE, Mai CT, Canfield MA, Rickard R, Wang Y, Meyer RE, et al. Updated national birth prevalence estimates for selected birth defects in the United States, 2004-2006. Birth Defects Res A Clin Mol Teratol. 2010 Dec;88(12):1008–16.
40. Medronho R de A, Bloch KV, Luiz RR, Werneck GL. Epidemiologia. 2a. São Paulo: Editora Atheneu; 2009. 685 p.
41. Kim J, Langlois PH, Herdt-Losavio ML, Agopian AJ. A Case-Control Study of Maternal Occupation and the Risk of Orofacial Clefts. J Occup Environ Med. 2016;58(8):833–9.
42. Wyszynski DF, Wu T. Use of US birth certificate data to estimate the risk of maternal cigarette smoking for oral clefting. Cleft Palate Craniofac J . 2002;39(2):188–92.
43. Carmichael SL, Ma C, Tinker SC, Rasmussen SA, Shaw GM, National Birth Defects Prevention Study. Maternal stressors and social support as risks for delivering babies with structural birth defects. HHS Public Access. 2014;28(4):338–44.

44. Wu Y, Liu B, Sun Y, Du Y, Santillan MK, Santillan DA, et al. Association of maternal prepregnancy diabetes and gestational diabetes mellitus with congenital anomalies of the newborn. Diabetes Care. 2020 Dec 1;43(12):2983–90.

45. Yang L, Wang H, Yang L, Zhao M, Guo Y, Bovet P, et al. Maternal cigarette smoking before or during pregnancy increases the risk of birth congenital anomalies: a population-based retrospective cohort study of 12 million mother-infant pairs. BMC Med. 2022 Dec 1;20(1).

46. Marinho F, Araújo VEM de, Porto DL, Ferreira HL, Coelho MRS, Lecca RCR, et al. Microcephaly in Brazil: prevalence and characterization of cases from the Information System on Live Births (Sinasc), 2000-2015. Epidemiologia e Serviços de Saúde [Internet]. 2016;25(4):701–12. Available from: http://www.iec.pa.gov.br/template_doi_ess.php?doi=10.5123/S1679-49742016000400701&scielo=S2237-96222016000400701

47. Calzolari E, Pierini A, Astolfi G, Bianchi F, Neville AJ, Rivieri F, et al. Associated Anomalies in Multi-Malformed Infants With Cleft Lip and Palate: An Epidemiologic Study of Nearly 6 Million Births in 23 EUROCAT Registries. Am J Hum Genet. 2007;221(3):212–21.

48. Wehby GL, Murray JC, Castilla EE, Lopez-Camelo JS, Ohsfeldt RL. Prenatal care demand and its effects on birth outcomes by birth defect status in Argentina. Econ Hum Biol. 2009;7(1):84–95.

6. CONSIDERAÇÕES FINAIS

A presente tese se propôs a avaliar fatores epidemiológicos, ambientais e genéticos relacionados às fissuras orofaciais, desordens orais potencialmente malignas e câncer oral em populações do Brasil e dos Estados Unidos. A partir dos resultados apresentados, foi possível concluir que:

- Com relação a tendência das pesquisas sobre a associação entre fissura orofacial e câncer, 70 documentos foram publicados entre 1977 e 2023. A maioria das publicações eram artigos do tipo caso-controle. *American Journal of Epidemiology* foi a fonte mais relevante. Vieira A, e Martelli Júnior H foram os principais autores nesta temática e maioria dos autores eram filiados principalmente à universidades do Brasil (Universidade de Montes Claros) e dos Estados Unidos (Universidade de Pittsburgh). O câncer gástrico, a leucemia e a câncer de mama foram os tipos de câncer mais estudados quanto a relação as fissuras orofaciais. A maioria das pesquisas quanto a temática de interesse incluiu crianças e adultos de ambos os sexos.
- O perfil epidemiológico dos nascidos vivos com FO no Brasil foi de indivíduos com FLP, do sexo masculino, de raça/cor branca, com peso normal e a termo, por cesariana e com índices de Apgar satisfatórios. Os casos de fissura foram mais frequentes entre mães que estavam na primeira e única gravidez e realizaram sete ou mais consultas de pré-natal. Essas mães tinham entre 20 e 29 anos, tinham de oito a onze anos de estudo e eram solteiras.
- A origem geográfica esteve associada ao tipo de fissura orofacial no Brasil, com maior razão de prevalência de ocorrência de fissura de lábio e/ou palato no interior em comparação à razão de prevalência de fissura de palato, que foi maior no litoral, sugerindo que desigualdades socioeconômicas entre as regiões poderiam atuar como estressores influenciando a expressão gênica por meio de alterações genéticas e/ou epigenéticas, alterando o risco para os tipos de FO.
- Achados deste estudo levam a sugerir que complicações perinatais (parto cesáreo, a morbidade materna, o nascimento prematuro, as condições anormais do recém-nascido, a dificuldade de amamentação, baixos índices de Apgar e o baixo peso ao nascer) foram desfechos mais frequentemente observados em gestantes que tiveram filhos com fissura em comparação a população geral de mulheres grávidas no Estados Unidos. Isso reforça a

hipótese que crianças com fissura orofacial estão mais propensas a problemas de saúde e fatores de risco que predispõem a ocorrência de complicações perinatais.

- Comparando os achados entre os países, potenciais fatores de risco e complicações gestacionais (idade materna, etnia materna, pré-natal, via de parto e prematuridade) foram mais expressivos para de crianças com fissura no brasil, sugerindo o papel dos determinantes sociais da saúde no aumento da suscetibilidade do indivíduo à fatores de risco para fissuras e condições precárias de saúde no nascimento da criança.
- Polimorfismos genéticos na via WNT (rs9879992/GSK3B, rs3923087/AXIN2 e; rs1533767/WNT11, rs392308/AXIN2, rs11867417/AXIN2) estiveram associados a ocorrência de desordens orais potencialmente malignas e ao carcinoma de células escamosas em populações do Brasil e Estados Unidos. Ainda, fatores ambientais, também relacionados ao risco de fissuras orofaciais, foram associados a ocorrência de desordens orais potencialmente malignas/carcinoma de células escamosas oral no Brasil. Os resultados deste estudo corroboram com sugestões de que fatores ambientais seriam capazes de interagir com os fatores biológicos predispondo a polimorfismos em genes da via WNT levando a ocorrência de fissuras orofaciais, quando estas alterações ocorrem durante o desenvolvimento embrionário, e ao câncer, quando estas ocorrem em outros estágios da vida.
- Além disso, variantes genéticas da via WNT (rs1533767/WNT11, rs3923087/AXIN2 e rs11867417/AXIN2) demosntram aumentar o risco para desordens orais potencialmente malignas e Carcinoma de Células Escamosas em indivíduos com perda dentária em uma população do Brasil, fortalecendo a ligação entre a saúde oral e doenças orais pré-malignas/malignas.
- Alterações de massa corpórea e comorbidades maternas (diabetes e hipertensão pré e gestacional) foram condições associadas a um risco aumentado de desenvolvimento de fissuras orofaciais nos Estados Unidos. Estes resultados, juntamente com o aumento da prevalência de obesidade, diabetes e hipertensão na população, reforçam a necessidade do monitoriamento da saúde da mulher antes e após a gestação.

REFERÊNCIAS

ABREU, M. H. N. G. DE et al. Temporal trend in the reported birth prevalence of cleft lip and/or cleft palate in Brazil, 2000 to 2013. **Birth Defects Research Part A - Clinical and Molecular Teratology**, v. 106, n. 9, p. 789–792, 2016.

ADAMS, A. K.; WISE-DRAPER, T. M.; WELLS, S. I. Human papillomavirus induced transformation in cervical and head and neck cancers. **Cancers**, v. 6, n. 3, p. 1793–1820, 2014.

AGBENORKU, P. Orofacial Clefts: A Worldwide Review of the Problem. **ISRN Plastic Surgery**, v. 2013, p. 1–7, 2013.

AGUIRRE-URIZAR, J. M.; LAFUENTE-IBÁÑEZ DE MENDOZA, I.; WARNAKULASURIYA, S. **Malignant transformation of oral leukoplakia: Systematic review and meta-analysis of the last 5 years**. **Oral Diseases**John Wiley and Sons Inc, , 1 nov. 2021.

AHMED SAKRAN, K. et al. **Analysis of Environmental Exposures for Nonsyndromic Cleft Lip and/or Palate: A Case-Control StudyIran J Public Health**. [s.l: s.n.]. Disponível em: <https://creativecommons.org/licenses/by-nc/4.0/>.

ALADE, A.; AWOTOYE, W.; BUTALI, A. Genetic and epigenetic studies in non-syndromic oral clefts. **Oral Diseases**, v. 28, n. 5, p. 1339–1350, 2021.

ALARCÓN, K. M. G.; SÁ, Á. J. DE A. Perfil epidemiológico dos pacientes portadores de fissuras labiopalatinas atendidos por equipe cirúrgica de referência no Estado do Amazonas. **Revista Brasileira de Cirurgia Plástica (RBCP) – Brazilian Journal of Plastic Sugery**, v. 32, n. 4, p. 486–490, 2017.

ALI, M. A. M.; HAMID, M. M. M. Risk Factors of Non-Syndromic Orofacial Clefts in Sudan during 2016-2017. **Annals of Medical and Health Sciences Research**, v. 9, n. 1, p. 472–477, 2019.

ALMEIDA, N. K. O.; ALMEIDA, R. M. V. R.; PEDREIRA, C. E. Adverse perinatal outcomes for advanced maternal age: A cross-sectional study of Brazilian births. **Jornal de Pediatria**, v. 91, n. 5, p. 493–498, 1 set. 2015.

ALTMANN, A. E.; HALLIDAY, J. L.; GILES, G. G. Associations between congenital malformations and childhood cancer. A register-based case-control study. **British Journal of Cancer**, v. 78, n. 9, p. 1244–1249, 1998.

ALVES, L. D. B.; MOURA, J. R.; FREITAS, V. S. Análise epidemiológica das fissuras labiais e/ou palatinas no município de Feira de Santana: estudo de corte transversal. **J Dent Pub H**, v. 10, n. 1, p. 128–139, 2019.

AMARASINGHE, H. K. et al. Diet and risk of oral potentially malignant disorders in rural Sri Lanka. **Journal of Oral Pathology & Medicine**, v. 42, n. 9, p. 656–662, 2013.

AMBROSIUS, W. T. **Methods in Molecular Biology**. Totowa, New Jersey, NJ: [s.n.]. v. 404

ANDRADE FILHO, P. A. et al. Insights from studies with oral cleft genes suggest associations between WNT-pathway genes and risk of oral cancer. **Journal of Dental Research**, v. 90, n. 6, p. 740–746, 2011.

ANDRADE, N. M. et al. Prevalence of cleft lip and palate in Brazilian children 2011 - 2015. **Internet Journal of Medical Update - EJOURNAL**, v. 13, n. 1, p. 10–14, 2018.

ANGULO-CASTRO, E. et al. Maternal risk factors associated with the development of cleft lip and cleft palate in Mexico: A case-control study. **Iranian Journal of Otorhinolaryngology**, v. 29, n. 4, p. 189–195, 2017.

ANTUNES, L. DOS S. et al. Risk factors for orofacial clefts: case-control study in non-syndromic individuals in Rio de Janeiro, Brazil. **Int J Dent**, v. 10, n. 1, p. 20–25, 2011.

ARIA, M.; CUCCURULLO, C. bibliometrix: An R-tool for comprehensive science mapping analysis. **Journal of Informetrics**, v. 11, n. 4, p. 959–975, 1 nov. 2017.

ASSIS, I. O. et al. IRE1 Less Common Homozygous Genotype in Families with Positive History of Cancer and Individuals Born with Cleft Lip and Palate. **Journal of Craniofacial Surgery**, p. 82, 2020.

BANAUDHA, K.; KUMAR, V.; VERMA, M. Challenges and Opportunities in Social Epigenomics and Cancer. Em: G., R.; VERMA, D. AND M. (Eds.). **Cancer Epigenetics for Precision Medicine: Methods and Protocols, Methods in Molecular Biology**. [s.l: s.n.]. v. 1856p. 114–128.

BARBER, E. L. et al. Indications contributing to the increasing cesarean delivery rate. **Obstetrics and Gynecology**, v. 118, n. 1, p. 29–38, jul. 2011.

BENUSIGLIO, P. R. et al. Cleft lip, cleft palate, hereditary diffuse gastric cancer and germline mutations in CDH1. **International Journal of Cancer**, v. 132, n. 10, p. 2470, 2013.

BERG, E. et al. Parental age and the risk of isolated cleft lip: A registry-based study. **Annals of Epidemiology**, v. 25, n. 12, p. 942- 947.e1, 1 dez. 2015.

BERNHEIM, N. et al. Embryology and epidemiology of cleft lip and palate. **B-Ent**, v. 2, n. SUPPL. 4, p. 11–19, 2006.

BETRÁN, A. P. et al. Trends and projections of caesarean section rates: Global and regional estimates. **BMJ Global Health**, v. 6, n. 6, p. 1–8, 2021.

BEZAMAT, M. et al. Phenome-Wide Scan Finds Potential Orofacial Risk Markers for Cancer. **Scientific Reports**, v. 10, n. 1, p. 1–10, 2020.

BEZAMAT, M.; ROTHENBERGER, S.; VIEIRA, A. R. Genetic contribution to cancer risk in patients with tooth loss: a genetic association study. **Scientific Reports**, v. 12, n. 1, p. 1–7, 2022.

BILLE, C. et al. Cancer risk in persons with oral cleft - A population-based study of 8,093 cases. **Am J Epidemiol**, v. 161, n. 11, p. 1047–1055, 2005.

BJØRGE, T. et al. Cancer risk in children with birth defects and in their families: A population based cohort study of 5.2 million children from Norway and Sweden. **Cancer Epidemiology Biomarkers and Prevention**, v. 17, n. 3, p. 500–506, 2008.

BLOT, W. J.; STILLER, C. A.; KINNIER-WILSON, L. M. Orofacial cleft and chidhood cancer. **Lancet**, v. 1, n. 8170, p. 722, 1980.

BOTTO, L. D. et al. Cancer Risk in Children and Adolescents with Birth Defects: A Population-Based Cohort Study. **PLoS ONE**, v. 8, n. 7, 2013.

BRASIL; SAÚDE, M. DA; SAÚDE, C. N. DE. **Resolução 466/2012**. , 2012.

BRENNAN, M. et al. Management of oral epithelial dysplasia: a review. **Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology**, v. 103, n. SUPPL., p. S19.e1-S19.e12, 2007.

BRESLOW, N. E.; BECKWITH, J. B. Epidemiological features of Wilms' tumor: Results of the national Wilms' tumor study. **Journal of the National Cancer Institute**, v. 68, n. 3, p. 429–436, 1982.

BUGTER, J. M.; FENDERICO, N.; MAURICE, M. M. Mutations and mechanisms of WNT pathway tumour suppressors in cancer. **Nature Reviews Cancer**, v. 21, n. 1, p. 5–21, 2021.

BUI, A. H. et al. Association Between Cleft Lip and/or Cleft Palate and Family History of Cancer. **Annals of plastic surgery**, v. 80, n. 4, p. S178–S181, 2018.

BULL, L. Genetics, Mutations, and Polymorphisms. Em: **NCBI Bookshelf**. Austin (TX): [s.n.]. p. 1–16.

CADONI, G. et al. A review of genetic epidemiology of head and neck cancer related to polymorphisms in metabolic genes, cell cycle control and alcohol metabolism. **Acta otorhinolaryngologica Italica : organo ufficiale della Societa italiana di otorinolaringologia e chirurgia cervico-facciale**, v. 32, n. 1, p. 1–11, 2012.

CARDOSO, E. et al. Nonsyndromic cleft lip and palate , gastric cancer and tooth agenesis. **Med Oral Patol Oral Cir Bucal**, v. 1, n. 23, p. e44-8, 2018.

CARMICHAEL, S. L.; MA, C.; SHAW, G. M. socioeconomic measures, orofacial clefts, and conotruncal heart defects in California. **Birth Defects Research Part A - Clinical and Molecular Teratology**, v. 85, n. 10, p. 850–857, 2009.

CAROZZA, S. E. et al. Are children with birth defects at higher risk of childhood cancers? **American Journal of Epidemiology**, v. 175, n. 12, p. 1217–1224, 2012.

CAVALCANTI, A. L. et al. An ecological study on orofacial clefts in northeastern Brazil. **Acta Facultatis Medicae Naissensis**, v. 36, n. 2, p. 102–109, 2019.

CENTERS FOR DISEASE CONTROL AND PREVENTION. **User Guide to the 2021 Natality Public Use File**. [s.l: s.n.].

CENTERS FOR DISEASE CONTROL AND PREVENTION. **Health Topics**. Disponível em: <https://www.cdc.gov/health-topics.html#cdc-atozlist>. Acesso em: 25 abr. 2023.

CHESHMI, B. et al. Assessment of the correlation between various risk factors and orofacial cleft disorder spectrum: a retrospective case-control study. **Maxillofacial Plastic and Reconstructive Surgery**, v. 42, n. 1, 2020.

CHRISTENSEN, K. et al. Long term follow up study of survival associated with cleft lip and palate at birth. **British Medical Journal**, v. 328, n. 7453, p. 1405–1406, 2004.

CLARK, J. D. et al. Socioeconomic status and orofacial clefts in Scotland, 1989 to 1998. **Cleft Palate-Craniofacial Journal**, v. 40, n. 5, p. 481–485, 2003.

CONWAY, D. I. et al. Socioeconomic inequalities and oral cancer risk: A systematic review and meta-analysis of case-control studies. **International Journal of Cancer**, v. 122, n. 12, p. 2811–2819, 2008.

CORREA, A. et al. Diabetes mellitus and birth defects. **American Journal of Obstetrics and Gynecology**, v. 199, n. 3, p. 237.e1-237.e9, 2008.

COSTA, R. DA; FARAH, R.; JACOBUCCI, G. Levantamento epidemiológico de fissuras labiopalatais no município de Maringá e região. **Rev assoc paul ciR dent**, v. 67, n. 1, p. 40–44, 2013.

COUTINHO, A. L. F. et al. Perfil epidemiológico dos portadores de fissuras orofaciais atendidos em um Centro de Referência do Nordeste do Brasil. **Revista Brasileira de Saude Materno Infantil**, v. 9, n. 2, p. 149–156, 2009.

CRUZ, L. A. **Mecanismos genéticos e epigenéticos na etiologia das fissuras orofaciais**. [s.l.] Instituto de Biociências da Universidade de São Paulo, 2017.

DAHIRI, B. et al. **Impact of pesticide exposure among rural and urban female population. An overview**. **International Journal of Environmental Research and Public Health**, 2021.

DAI, L. et al. Time Trends in Oral Clefts in Chinese Newborns: Data From the Chinese National Birth Defects Monitoring Network. **Birth Defects Res A Clin Mol Teratol.**, v. 88, n. 1, p. 41–47, 2010.

DE CARVALHO, P. H. P. et al. Parental age is related to the occurrence of cleft lip and palate in Brazilian populations. **Brazilian Journal of Oral Sciences**, v. 15, n. 2, p. 167–170, 2016.

DEELEY, K.; NOEL, J.; VIEIRA, A. R. Comparative Study of Five Commercially Available Saliva Collection Kits for DNA Extraction. **Clin Lab**, v. 62(9), n. 9, p. 1809–1813, 2016.

DE FREITAS, E. M. et al. Polymorphisms associated with oral clefts as potential susceptibility markers for oral and breast cancer. **Archives of Oral Biology**, v. 99, n. December 2018, p. 9–14, 2019.

DEROO, L. A.; GAUDINO, J. A.; EDMONDS, L. D. Orofacial cleft malformations: Associations with maternal and infant characteristics in Washington State. **Birth Defects Research Part A - Clinical and Molecular Teratology**, v. 67, n. 9, p. 637–642, 2003.

DIAS, V. O. et al. Nonsyndromic oral cleft in first-degree relatives of patients with acute lymphoblastic leukemia. **Dentistry Journal**, v. 8, n. 1, p. 6–11, 2020.

DIEN, V. H. A. et al. Maternal exposures and risk of oral clefts in South Vietnam. **Birth Defects Research**, v. 110, n. 6, p. 527–537, 3 abr. 2018.

DIETZ, A. et al. Risk of breast cancer in families with cleft lip and palate. **Annals of Epidemiology**, v. 22, n. 1, p. 37–42, 2012.

DI NINNO, C. Q. DE M. S. et al. Levantamento epidemiológico dos pacientes portadores de fissura de lábio e/ou palato de um centro especializado de Belo Horizonte. **Revista CEFAC**, v. 13, n. 6, p. 1002–1008, 2011.

DIXON, M. J. et al. Cleft lip and palate: synthesizing genetic and environmental influences. **NIH Public Access**, v. 3, n. 3, p. 203, 2011.

DONKOR, P.; PLANGE-RHULE, G.; AMPONSAH, E. K. A prospective surgery of patients with cleft lip and palate in kumasi. **West African Journal of Medicine**, 2007.

DONTHU, N. et al. How to conduct a bibliometric analysis: An overview and guidelines. **Journal of Business Research**, v. 133, p. 285–296, 1 set. 2021.

DVIVEDI, J.; DVIVEDI, S. A clinical and demographic profile of the cleft lip and palate in Sub-Himalayan India: A hospital-based study. **Indian Journal of Plastic Surgery**, v. 45, n. 1, p. 115–120, 2012.

EHLI, E. A. et al. Using a commercially available DNA extraction kit to obtain high quality human genomic DNA suitable for PCR and genotyping from 11-year-old saliva saturated cotton spit wads. **BMC Research Notes**, v. 1, p. 1–4, 2008.

EJAZ, I.; GHAFOOR, S. Wnt Signaling Pathway in Oral Lesions. **Journal of the Pakistan Medical Association**, v. 69, n. 11, p. 1687–1692, 2019.

ERIKSSON, U. J.; CEDERBERG, J.; WENTZEL, P. Congenital malformations in offspring of diabetic mothers - Animal and human studies. **Reviews in Endocrine and Metabolic Disorders**, v. 4, n. 1, p. 79–93, 2003.

ESCOFFIÉ-RAMIREZ, M.; ET AL. Asociación de labio y/o paladar hendido com variables de posición socioeconómica: un estudio de casos y controles. **Rev Bras Saúde Maten Infant**, v. 10, n. 3, p. 323–9, 2010.

ESHETE, M. et al. The Role of Environmental Factors in the Etiology of Nonsyndromic Orofacial Clefts. **Journal of Craniofacial Surgery**, v. 31, n. 1, p. 113–116, 2020.

FANG, M. et al. Association of Five Snps in Cytotoxic T-Lymphocyte Antigen 4 and Cancer Susceptibility: Evidence from 67 Studies. **Cellular Physiology and Biochemistry**, v. 47, n. 1, p. 414–427, 2018.

FAN, R. G.; PORTUGUEZ, M. W.; NUNES, M. L. Cognition, behavior and social competence of preterm low birth weight children at school age. **Clinics**, v. 68, n. 7, p. 915–921, jul. 2013.

FARIA, Á. C. O. **Fatores de susceptibilidade às fissuras orofaciais**. [s.l.] Universidade de São Paulo, 2019.

FARINHAS, G. V. **Os sentidos produzidos nos discursos de mães frente ao nascimento de filhos com malformação labiopalatal e os recursos da rede do SUS disponibilizados para o cuidado**. Dissertação—[s.l.] Universidade de Santa Cruz do Sul, 2017.

FELL, M. et al. Maternal Cigarette Smoking and Cleft Lip and Palate: A Systematic Review and Meta-Analysis. **Cleft Palate-Craniofacial Journal**, v. 59, n. 9, p. 1185–1200, 2022.

FIGUEIRÊDO, C. J. R. et al. Prevalence of oral clefts in the State of Rio Grande do Norte, Brazil , between 2000-2005. **Rev Paul Pediatr.**, v. 29, n. 1, p. 29–34, 2011.

FIGUEIREDO, J. C. et al. Parental risk factors for oral clefts among Central Africans, Southeast Asians, and Central Americans. **Birth Defects Research Part A - Clinical and Molecular Teratology**, v. 103, n. 10, p. 863–879, 2015.

FISHER, P. G. et al. Cancer in children with nonchromosomal birth defects. **Journal of Pediatrics**, v. 160, n. 6, p. 978–983, 2012.

FREITAS, E. M. DE et al. Polymorphisms associated with oral clefts as potential susceptibility markers for oral and breast cancer. **Archives of Oral Biology**, v. 99, n. 2019, p. 9–14, 2019.

FREITAS, M. DA C. A. et al. Estudo epidemiológico das fissuras labiopalatinas na Bahia. **Revista UNINGÁ, Maringá - PR**, n. 37, p. 13–21, 2013.

GARLAND, M. A. et al. Role of epigenetics and miRNAs in orofacial clefts. **Birth Defects Research**, v. 112, n. 19, p. 1635–1659, 2020.

GARLAND, M. A.; REYNOLDS, K.; ZHOU, C. J. Environmental mechanisms of orofacial clefts. **Birth Defects Research**, p. 1–39, 2020.

GOLALIPOUR M J et al. Maternal Risk Factors for Oral Clefts- A Case-Control Study. **Iranian Journal of Otorhinolaryngology**, v. 24, n. 4, 2012.

GOLD, E. B. et al. The role of family history in risk of childhood brain tumors. **Cancer**, v. 73, n. 4, p. 1302–1311, 1994.

GONÇALVES, E. et al. Risk of leukemia in first degree relatives of patients with nonsyndromic cleft lip and palate. **Brazilian oral research**, v. 28, n. 1, p. 1–3, 2014.

GONZÁLEZ-OSORIO, C. A. et al. Estudio ecológico en México (2003-2009) sobre labio y/o paladar hendido y factores sociodemográficos, socioeconómicos y de contaminación asociados. **Anales de Pediatria**, v. 74, n. 6, p. 377–387, 2011.

GRÜNEBAUM, A. et al. Neonatal mortality in the United States is related to location of birth (hospital versus home) rather than the type of birth attendant. **American Journal of Obstetrics and Gynecology**, v. 223, n. 2, p. 254.e1-254.e8, 1 ago. 2020.

GUNTINAS-LICHIUS, O. et al. Head and neck cancer in Germany: A site-specific analysis of survival of the Thuringian cancer registration database. **Journal of Cancer Research and Clinical Oncology**, v. 136, n. 1, p. 55–63, 2010.

GUPTA, B.; JOHNSON, N. W.; KUMAR, N. Global Epidemiology of Head and Neck Cancers: A Continuing Challenge. **Oncology (Switzerland)**, v. 91, n. 1, p. 13–23, 2016.

HAO, Y. et al. Association of parental environmental exposures and supplementation intake with risk of nonsyndromic orofacial clefts: A case-control study in Heilongjiang Province, China. **Nutrients**, v. 7, n. 9, p. 7172–7184, 2015.

HASHIBE, M. et al. Interaction between tobacco and alcohol use and the risk of head and neck cancer: pooled analysis in the INHANCE consortium. **NIH Public Access**, v. 18, n. 2, p. 541–550, 2009.

HEIST, R. S. et al. MDM2 polymorphism, survival, and histology in early-stage non-small-cell lung cancer. **Journal of Clinical Oncology**, v. 25, n. 16, p. 2243–2247, 2007.

HERKRATH, A. P. C. D. Q. et al. Parental age as a risk factor for non-syndromic oral clefts: A meta-analysis. **Journal of Dentistry**, v. 40, n. 1, p. 3–14, 2012.

HINDLE, I. et al. Is alcohol responsible for more intra-oral cancer? **Oral Oncology**, v. 36, n. 4, p. 328–333, 2000.

HOCHMAN, B. et al. Desenhos de pesquisa. **Acta Cirúrgica Brasileira**, v. 20, n. 2, 2005.

HOLMES, L. B. The AED (Antiepileptic Drug) Pregnancy Registry. **Archives of Neurology**, v. 61, n. 5, p. 673, 2004.

HOPKINS, J. et al. Genetic polymorphisms and head and neck cancer outcomes: A review. **Cancer Epidemiology Biomarkers and Prevention**, v. 17, n. 3, p. 490–499, 2008.

HOZYASZ, K. K. et al. Relation between the concentration of zinc in maternal whole blood and the risk of an infant being born with an orofacial cleft. **British Journal of Oral and Maxillofacial Surgery**, v. 47, n. 6, p. 466–469, 2009.

HUANG, Y.-D. et al. Screening of differentially expressed genes related to esophageal squamous cell carcinoma and functional analysis with DNA microarrays. **Asian Pacific Journal of Cancer Prevention**, v. 12, p. 4553–4557, 2013.

IBGE - INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA. **Panorama Censo 2022**. [s.l: s.n.]. Disponível em: <https://censo2022.ibge.gov.br/panorama/?utm_source=ibge&utm_medium=home&utm_campaign=portal>.

INCHINGOLO, A. M. et al. Modifiable Risk Factors of Non-Syndromic Orofacial Clefts : A Systematic Review. **Children**, v. 9, n. 12, p. 1846, 2022.

IPDTOC WORKING GROUP. Prevalence at birth of cleft lip with or without cleft palate: Data from the International Perinatal Database of Typical Oral Clefts (IPDTOC). **Cleft Palate-Craniofacial Journal**, v. 48, n. 1, p. 66–81, 2011.

IZEDONMWEN, O. M.; CUNNINGHAM, C.; MACFARLANE, T. V. What is the Risk of Having Offspring with Cleft Lip/Palate in Pre-Maternal Obese/Overweight Women When Compared to Pre-Maternal Normal Weight Women? A Systematic Review and Meta-Analysis. **Journal of Oral and Maxillofacial Research**, v. 6, n. 1, 31 mar. 2015.

IZZO, J. G. et al. Cyclin D1 guanine/adenine 870 polymorphism with altered protein expression is associated with genomic instability and aggressive clinical biology of esophageal adenocarcinoma. **Journal of Clinical Oncology**, v. 25, n. 6, p. 698–707, 2007.

JAMES, O. et al. Parental age and the risk of cleft lip and palate in a Nigerian population - A case-control study. **Annals of Maxillofacial Surgery**, v. 10, n. 2, p. 429–433, 1 jul. 2020.

JAYARAJAN, R.; NATARAJAN, A.; NAGAMUTTU, R. Efficacy of Periconceptional High-Dose Folic Acid in Isolated Orofacial Cleft Prevention: A Systematic Review. **Indian Journal of Plastic Surgery**, v. 52, n. 2, p. 153–159, 2019.

JINDAL, A.; VIEIRA, A. R. Family history of cleft lip and palate in subjects diagnosed with leukemia. **American Journal of Medical Genetics, Part A**, v. 158 A, n. 3, p. 678–679, 2012.

JOHANSEN, A. M. W. et al. Maternal dietary intake of vitamin A and risk of orofacial clefts: A population-based case-control study in Norway. **American Journal of Epidemiology**, v. 167, n. 10, p. 1164–1170, 2008.

JOHNSON, D. E. et al. Head and neck squamous cell carcinoma. **Nature Reviews Disease Primers**, v. 6, n. 1, 2020.

KADIR, A. et al. Systematic review and meta-analysis of the birth prevalence of orofacial clefts in low- and middle-income countries. **Cleft Palate-Craniofacial Journal**, v. 54, n. 5, p. 571–581, 2017.

KAPOS, F. P. et al. Risk of non-syndromic orofacial clefts by maternal rural-urban residence and race/ethnicity: A population-based case-control study in Washington State 1989-2014. **Paediatric and Perinatal Epidemiology**, v. 35, n. 3, p. 292–301, 2021.

KARIM, R. Z. et al. The significance of the Wnt pathway in the pathology of human cancers. **Pathology**, v. 36, n. 2, p. 120–128, 1 abr. 2004.

KHAN, A. M. I.; PRASHANTH, C.; SRINATH, N. Genetic etiology of cleft lip and cleft palate. **AIMS Molecular Science**, v. 7, n. 4, p. 328–348, 2020.

KHARE, P. et al. Quantitative and qualitative assessment of DNA extracted from saliva for its use in forensic identification. **Journal of Forensic Dental Sciences**, v. 6, n. 2, p. 81, 2014.

KING, J. C. Maternal obesity, metabolism, and pregnancy outcomes. **Annu Rev Nutr**, v. 26, p. 271–291, 2006.

KLUIJT, I. et al. CDH1-related hereditary diffuse gastric cancer syndrome: Clinical variations and implications for counseling. **International Journal of Cancer**, v. 131, n. 2, p. 367–376, 2012.

KOMIYA, Y.; HABAS, R. Wnt signal transduction pathways. **Organogenesis**, v. 4, n. 2, p. 68–75, 2008.

KOZMA, A. Gestational Diabetes Mellitus and the Development of Cleft Lip / Palate in Newborns. **Acta Endocrinologica**, v. 15, n. 1, p. 118–122, 2019.

KRAPELS, I. P. C. et al. Periconceptional health and lifestyle factors of both parents affect the risk of live-born children with orofacial clefts. **Birth Defects Research Part A - Clinical and Molecular Teratology**, v. 76, n. 8, p. 613–620, 2006.

KROST, B.; SCHUBERT, J. Influence of season on prevalence of cleft lip and palate. **International Journal of Oral and Maxillofacial Surgery**, v. 35, n. 3, 2006.

KRUPPA, K. et al. Cleft Lip and/or Palate and Associated Risks in Lower-Middle-Income Countries: A Systematic Review. **Cleft Palate-Craniofacial Journal**, p. 1–9, 2021.

KÜCHLER, E. C. et al. Tooth agenesis association with self-reported family history of cancer. **Journal of Dental Research**, v. 92, n. 2, p. 149–155, 2013.

KUTBI, H. et al. Maternal underweight and obesity and risk of orofacial clefts in a large international consortium of population-based studies. **International Journal of Epidemiology**, v. 46, n. 1, p. 190–199, 2017.

KUTBI, H. A. **The Role of Obesity, Diabetes, and Hypertension in Cleft lip and Cleft Palate Birth Defects**. Logan: Utah State University, 2014.

LAMMER, E. J. et al. Maternal smoking and the risk of orofacial clefts: Susceptibility with NAT1 and NAT2 polymorphisms. **Epidemiology**, v. 15, n. 2, p. 150–156, 2004.

LEI, R. et al. Population-Based Study of Birth Prevalence and Factors Associated with Cleft Lip and / or Palate in Taiwan 2002 – 2009. **PLoS ONE**, v. 8, n. 3, p. 1–5, 2013.

LESLIE, E. J. et al. Genome-wide meta-analyses of nonsyndromic orofacial clefts identify novel associations between FOXE1 and all orofacial clefts, and TP63 and cleft lip with or without cleft palate. **HHS Public Access**, v. 136, n. 3, p. 275–286, 2017.

LESLIE, E. J. Genetic models and approaches to study orofacial clefts. **Oral Diseases**, v. 28, n. 5, p. 1327–1338, 2022.

LESLIE, E. J.; MARAZITA, M. L. Genetics of cleft lip and cleft palate. **American Journal of Medical Genetics, Part C: Seminars in Medical Genetics**, v. 163, n. 4, p. 246–258, 2013.

LETRA, A. et al. AXIN2 and CDH1 polymorphisms, tooth agenesis, and oral clefts. **Birth Defects Research Part A - Clinical and Molecular Teratology**, v. 85, n. 2, p. 169–173, 2009.

LIMA, L. S.; SILVÉRIO, M. D. O.; OLIVEIRA, M. S. Frequency of Cancer in First-Degree Relatives of Patients with Cleft Lip and / or Palate in the Brazilian Population. **Braz Dent J**, v. 24, p. 200–203, 2013.

LIU, F.; MILLAR, S. E. Wnt/β-catenin signaling in oral tissue development and disease. **Journal of Dental Research**, v. 89, n. 4, p. 318–330, 2010.

LIU, Y. **Clinicopathologic factors for disease-free survival inearly stage orall squamous cell carcinoma**. [s.l.] University of Pittsburgh, 2018.

LI, X. et al. New insights of the correlation between AXIN2 polymorphism and cancer risk and susceptibility: evidence from 72 studies. **BMC Cancer**, v. 21, n. 1, p. 1–15, 2021.

LUDWIG, K. U. et al. Genome-wide meta-analyses of nonsyndromic cleft lip with or without cleft palate identify six new risk loci. **NIH Public Access**, v. 44, n. 9, p. 968–971, 2012.

LUIZ, A. F.; RODRIGUES, L. P. G. D'ARCE; DEMARCO, N. R. Influência da sazonalidade na incidência de fissuras labiopalatinas em um centro de referência no Oeste do Paraná. **Research, Society and Development**, v. 11, n. 2, 2022.

LUO, Y. L. et al. Maternal age, parity and isolated birth defects: A population-based case-control study in Shenzhen, China. **PLoS ONE**, v. 8, n. 11, p. 4–9, 2013.

LUPO, P. J. et al. Neighborhood-based socioeconomic position and risk of oral clefts among offspring. **American Journal of Public Health**, v. 105, n. 12, p. 2518–2525, 2015.

LY, S. et al. Paternal risk factors for oral clefts in northern Africans, southeast Asians, and central Americans. **International Journal of Environmental Research and Public Health**, v. 14, n. 6, p. 1–12, 2017.

MACHADO, R. A. et al. Potential genetic markers for nonsyndromic oral clefts in the Brazilian population: A systematic review and meta-analysis. **Birth Defects Research**, v. 110, n. 10, p. 827–839, 2018.

MACHADO, R. A.; POPOFF, D. A. V.; MARTELLI-JÚNIOR, H. Relationship between non-syndromic oral clefts and cancer: a systematic review and meta-analysis. **Oral Diseases**, 2022.

MAI, C. T. et al. National population-based estimates for major birth defects, 2010–2014. **HHS Public Access**, v. 111, n. 18, p. 1420–1435, 2019.

MALIC, C. C. et al. Incidence, Risk Factors, and Mortality Associated with Orofacial Cleft among Children in Ontario, Canada. **JAMA**, v. 3, n. 2, p. 1–13, 2020.

MANCILLA, V. J. et al. Understanding the Interplay Between Health Disparities and Epigenomics. **Frontiers in Genetics**, v. 11, n. August, p. 1–14, 2020.

MANN, J. R. et al. Congenital abnormalities in children with cancer and their relatives: results from a case-control study (IRESCC*). **British Journal of Cancer**, v. 68, n. 2, p. 357–363, 1993.

MARAZITA, M. L. The evolution of human genetic studies of cleft lip and cleft palate. **Annual Review of Genomics and Human Genetics**, v. 13, p. 263–283, 2012.

MARAZITA, M. L.; MOONEY, M. P. Current concepts in the embryology and genetics of cleft lip and cleft palate. **Clinics in Plastic Surgery**, v. 31, n. 2, p. 125–140, 2004.

MARKOV, D.; JACQUEMYN, Y.; LEROY, Y. Bilateral cleft lip and palate associated with increased nuchal translucency and maternal cocaine abuse at 14 weeks of gestation. **Clinical and Experimental Obstetrics and Gynecology**, v. 30, n. 2–3, p. 109–110, 2003.

MARTELLI, D. R. B. et al. Risk of nonsyndromic cleft lip and palate in relatives of women with breast cancer. **American Journal of Medical Genetics, Part A**, v. 164, n. 1, p. 270–271, 2014.

MARTINEZ-BALIBREA, E. et al. Combined analysis of genetic polymorphisms in thymidylate synthase, uridine diphosphate glucoronosyltransferase and X-ray cross complementing factor 1 genes as a prognostic factor in advanced colorectal cancer patients treated with 5-fluorouracil plus oxa. **Oncology Reports**, v. 17, n. 3, p. 637–645, 2007.

MATOS, F. G. DE O. A. et al. Perfil epidemiológico das fissuras labiopalatais de crianças atendidas em um centro de referência paranaense. **Rev. Enferm. UFSM**, v. 10, n. 28, p. 1–14, 2020.

MCBRIDE, W. A. et al. Subphenotyping and classification of orofacial clefts: Need for orofacial cleft subphenotyping calls for revised classification. **Cleft Palate-Craniofacial Journal**, v. 53, n. 5, p. 539–549, 2016.

MCMAHON, S.; CHEN, A. Y. Head and neck cancer. **Medical Clinics of North America**, v. 22, p. 21–24, 2003.

MEDRONHO, R. DE A. et al. **Epidemiologia**. 2nd. ed. São Paulo: Atheneu, 2009.

MENEZES, R. et al. AXIN2, Orofacial Clefts and Positive Family History for Cancer. **J Am Dent Assoc**, v. 140, n. 1, p. 80–84, 2009.

MENON, R. S. **Wnt Signaling in Oral Cancer Initiating Cells**. Doctoral dissertation—[s.l.] Harvard School of Dental Medicine, 2017.

MERTENS, A. C. et al. Congenital abnormalities in children with acute leukemia: A report from the Children's Cancer Group. **Journal of Pediatrics**, v. 133, n. 5, p. 617–623, 1998.

MESSER, L. C. et al. Urban-Rural Residence and the Occurrence of Cleft Lip and Cleft Palate in Texas, 1999-2003. **Annals of Epidemiology**, v. 20, n. 1, p. 32–39, 2010.

MICHALSKI, A. M. et al. Sex ratios among infants with birth defects, National Birth Defects Prevention Study, 1997-2009. **American Journal of Medical Genetics**, v. 167, n. 5, p. 1071–1081, 2015.

Ministério da Saúde Instituto Nacional de Câncer José Alencar Gomes da Silva Ministério da Saúde Instituto Nacional de Câncer. . [s.l: s.n.].

MOHAMMED, S. I.; SPRINGFIELD, S.; DAS, R. Role of epigenetics in cancer health disparities. **Methods in Molecular Biology**, v. 863, p. 395–410, 2012.

MONLLEÓ, I. L. et al. Implementing the Brazilian Database on Orofacial Clefts. **Plastic Surgery International**, v. 2013, n. March, p. 1–10, 2013.

MONLLEÓ, I. L. et al. Diagnostic implications of associated defects in patients with typical orofacial clefts. **Jornal de Pediatria**, v. 91, n. 5, p. 485–492, 2015.

MONLLEÓ, I. L.; MOSSEY, P. A.; GIL-DA-SILVA-LOPES, V. L. Evaluation of craniofacial care outside the brazilian reference network for craniofacial treatment. **Cleft Palate-Craniofacial Journal**, v. 46, n. 2, p. 204–211, 2009.

MONTEIRO, J. L. G. C. et al. **Five decades of orofacial cleft management and research in BrazilInternational Journal of Oral & Maxillofacial Surgery**. [s.l.] International Association of Oral and Maxillofacial Surgery, 2018.

MOSSEY, P. A. et al. Cleft lip and palate. **The Lancet**, v. 374, p. 1773–1785, 2009.

MOSSEY, P. P. A.; MODELL, B. Epidemiology of Oral Clefts 2012: An International Perspective. **Epidemiology, Aetiology and Treatment. Front Oral Biol.**, v. 16, p. 1–18, 2012.

MOURA, J. R. **Perfil Clínico-Epidemiológico dos portadores de Fissuras Orofaciais em residentes do estado da Bahia: um estudo descritivo**. [s.l.] Universidade Estadual de Feira de Santana, 2014.

MUKHOPADHYAY, N. et al. Genome-Wide Association Study of Non-syndromic Orofacial Clefts in a Multiethnic Sample of Families and Controls Identifies Novel Regions. **Frontiers in Cell and Developmental Biology**, v. 9, 2021.

MULLEN, M. C. et al. Racial and Ethnic Disparities in Primary Cleft Lip and Cleft Palate Repair. **Cleft Palate-Craniofacial Journal**, v. 60, n. 4, 2023.

MURRAY, J. C. Gene/environment causes of cleft lip and/or palate. **Clinical Genetics**, v. 61, n. 4, p. 248–256, 2002.

MURRAY, J. C.; WEHBY, G. L. Folic acid and orofacial clefts: A review of the evidence. **Oral Diseases**, v. 16, n. 1, p. 11–19, 2010.

NADER, N. H.-R.; GATAA, I. S.; MOHAMMAD, D. N. CD34 and Wnt3 Expression in Potentially Malignant Oral Disorders. **Journal of Baghdad College of Dentistry**, v. 29, n. 3, p. 59–67, 2017.

NAGAPPAN, N.; JOHN, J. Sociodemographic profile of orofacial cleft patients in India: A hospital-based study. **International Journal of Medicine and Public Health**, v. 5, n. 1, p. 35, 2015.

NAROD, S. A. et al. Congenital anomalies and childhood cancer in Great Britain. **American Journal of Human Genetics**, v. 60, n. 3, p. 474–485, 1997.

NASREDDINE, G.; EL HAJJ, J.; GHASSIBE-SABBAGH, M. Orofacial clefts embryology, classification, epidemiology, and genetics. **Mutation Research**, v. 787, p. 108373, 2021.

NEHRA, R. A. et al. How Low Socioeconomic Status during Pregnancy Causes Orofacial Clefts in Newborns. **P J M H S**, v. 15, n. 2, p. 432–433, 2021.

NISHI, M. et al. Congenital Malformations and Childhood Cancer. **Medical and Pediatric Oncology**, v. 34, p. 250–254, 2000.

NIU, Y. M. et al. Increased risks between Interleukin-10 gene polymorphisms and haplotype and head and neck cancer: A meta-analysis. **Scientific Reports**, v. 5, n. June, p. 1–10, 2015.

NOOROLLAHIAN, M. et al. Cleft lip and palate and related factors: A 10 years study in university hospitalised patients at Mashhad - Iran. **African Journal of Paediatric Surgery**, v. 12, n. 4, p. 286–290, 1 out. 2015.

NOTTERMAN, D. A.; MITCHELL, C. Epigenetics and Understanding the Impact of Social Determinants of Health. **HHS Public Access**, v. 62, n. 5, p. 1227–1240, 2015.

NUNES, A. P. et al. Quality of DNA extracted from saliva samples collected with the Oragene DNA self-collection kit. **BMC Medical Research Methodology**, v. 12, 2012.

NYARKO, K. A. et al. Does the relationship between prenatal care and birth weight vary by oral clefts? Evidence using South American and united states samples. **Journal of Pediatrics**, v. 162, n. 1, 2013.

OBERMAIR, F. et al. Cleft lip/palate and hereditary diffuse gastric cancer: report of a family harboring a CDH1 c.687 + 1G > A germline mutation and review of the literature. **Familial Cancer**, v. 18, n. 2, p. 253–260, 2019.

OLIVEIRA, M. M. DE et al. Avaliação do Sistema de Informações sobre Nascidos Vivos. Brasil, 2006 a 2010. **Epidemiologia e Serviços de Saúde**, v. 24, n. 4, p. 629–640, 2015.

OLIVEIRA, M. A.; VELLARDE, G. C.; SÁ, R. A. M. Entendendo a pesquisa clínica IV: estudos de caso controle. **Femina**, v. 43, n. 4, p. 2–7, 2015.

PANDESHWAR, P.; DAS, R. Role of oral fluids in DNA investigations. **Journal of Forensic and Legal Medicine**, v. 22, p. 45–50, 2014.

PECK, C. J. **A Nationwide Assessment Of Disparities In Primary Cleft Lip A Nationwide Assessment Of Disparities In Primary Cleft Lip Repair Repair**. [s.l: s.n.]. Disponível em: <https://elischolar.library.yale.edu/ymtdl/4112>.

Pittsburgh Orofacial Cleft Studies. Disponível em: <https://www.dental.pitt.edu/research/ccdg/participate-research/pittsburgh-orofacial-cleft-studies>.

POLGÁROVÁ, K.; BEHULIAK, M.; CELEC, P. Effect of saliva processing on bacterial DNA extraction. **New Microbiologica**, v. 33, n. 4, p. 373–379, 2010.

POPOFF, D. A. V. et al. Non-syndromic oral clefts and risk of cancer: a systematic review. **Dentistry 3000**, v. 1, n. 1, p. 12–18, 2013.

PRADAT, P. et al. First Trimester Exposure to Corticosteroids and Oral Clefts. **Birth Defects Research Part A - Clinical and Molecular Teratology**, v. 67, n. 12, p. 968–970, 2003.

PUHÓ, E. H. et al. Drug treatment during pregnancy and isolated orofacial clefts in Hungary. **Cleft Palate-Craniofacial Journal**, v. 44, n. 2, p. 194–202, 2007.

PURCELL, S. et al. PLINK: A tool set for whole-genome association and population-based linkage analyses. **American Journal of Human Genetics**, v. 81, n. 3, p. 559–575, 2007.

PURWANINGSIH, N. M. S. et al. Wnt pathway in oral cancer: A review update. **Saudi Dental Journal**, v. 33, n. 8, p. 813–818, 2021.

PYTYNIA, K. B.; DAHLSTROM, K. R.; STURGIS, E. M. Epidemiology of HPV-associated oropharyngeal cancer. **HHS Public Access**, v. 50, n. 5, p. 380–386, 2014.

QUINQUE, D. et al. Evaluation of saliva as a source of human DNA for population and association studies. **Analytical Biochemistry**, v. 353, n. 2, p. 272–277, 2006.

RAHMAN, Q. B. et al. Global Burden of Head and Neck Cancer. **Oral and Maxillofacial Surgery Clinics of North America**, v. 32, n. 3, p. 367–375, 2020.

RAMOS, N. et al. Biorrepositório de saliva em estudos genetico-moleculares: avaliacao de diferentes protocolos de extracao de DNA apos longos periodos de armazenamento. Em: **Análise Crítica das Ciências Biológicas e da Natureza 2**. Ponta Grossa PR: Atena Editora, 2019. v. 2.

RANADE, K. et al. High-throughput genotyping with single nucleotide polymorphisms. **Genome Research**, v. 11, n. 7, p. 1262–1268, 2001.

RANGANATHAN, K.; KAVITHA, L. Oral epithelial dysplasia: Classifications and clinical relevance in risk assessment of oral potentially malignant disorders. **Journal of oral and Maxillofacial Pathology**, v. 23, n. 1, p. 19–27, 2019.

RANKIN, J. et al. Maternal body mass index and congenital anomaly risk: A cohort study. **International Journal of Obesity**, v. 34, n. 9, p. 1371–1380, 2010.

RASMUSSEN, S. A. et al. Guidelines for case classification for the National Birth Defects Prevention Study. **Birth Defects Research Part A - Clinical and Molecular Teratology**, v. 67, n. 3, p. 193–201, 2003.

RAUT, J. R. et al. Proportion of Orofacial Clefts Attributable to Recognized Risk Factors. **Physiology & behavior**, v. 56, n. 2, p. 151–158, 2019.

REGINA ALTOÉ, S. et al. Influence of Parental Exposure to Risk Factors in the Occurrence of Oral Clefts. **Journal of Dentistry**, v. 21, n. 2, p. 119–126, 2020.

REIBEL, J. et al. Oral potentially malignant disorders and oral epithelial dysplasia. Em: EL-NAGGAR AK, CHAN JKC, GRANDIS JR, TAKATA T, S. P. (Ed.). **WHO classification of tumours of the head and neck.** 4th. ed. Lyon: IARC Press, 2017.

REYES, M. et al. Increased nuclear β-catenin expression in oral potentially malignant lesions: A marker of epithelial dysplasia. **Medicina Oral, Patologia Oral y Cirugia Bucal**, v. 20, n. 5, p. e540–e546, 2015.

REYES, M. et al. Nuclear localization of β-catenin and expression of target genes are associated with increased Wnt secretion in oral dysplasia. **Oral Oncology**, n. 94, p. 58–67, 2019.

REYNOLDS, K. et al. Genetics and signaling mechanisms of orofacial clefts. **Birth Defects Research**, v. 112, n. 19, p. 1588–1634, 2020.

RODRIGUES, K. et al. Prevalence of orofacial clefts and social factors in Brazil. **Brazilian Oral Research**, v. 23, n. 1, p. 38–42, 2009.

RODRIGUES, K. M. D. et al. Relationship between the number of prenatal care visits and the occurrence of adverse perinatal outcomes. **Revista da Associacao Medica Brasileira**, v. 68, n. 2, p. 256–260, 2022.

RODRIGUES, L. DOS S. et al. Características das crianças nascidas com malformações congênitas no município de São Luís, Maranhão, 2002-2011. **Epidemiologia e Serviços de Saúde**, v. 23, n. 2, p. 295–304, 2014.

ROMERO, D. E.; CUNHA, C. B. Disponibilidade e qualidade da informação epidemiológica e demográfica no Sistema de Informações sobre Nascidos Vivos (SINASC) e no Sistema de Informação de Mortalidade (SIM) para menores de um. **Anais ABEP**, n. Xv, p. 1–18, 2006.

ROMERO, D. E.; CUNHA, C. B. Avaliação da qualidade das variáveis epidemiológicas e demográficas do Sistema de Informações sobre Nascidos Vivos , 2002. **Cad. Saúde Públic**, v. 23, n. 3, p. 701–714, 2007.

ROUQUAYROL, M. Z.; ALMEIDA-FILHO, N. DE. **Epidemiologia & Saúde**. 6. ed. de Janeiro: Medsi, 2003.

RYLANDS, J.; LOWE, D.; ROGERS, S. N. Outcomes by area of residence deprivation in a cohort of oral cancer patients: Survival, health-related quality of life, and place of death. **Oral Oncology**, v. 52, p. 30–36, 2016.

RYU, J. Y. et al. The prevalence, risk of premature births, mortality and causes of death of cleft lip with or without palate in South Korea: a nationwide population-based cohort study. **International Journal of Epidemiology**, v. 51, n. 3, p. 974–983, 1 jun. 2022.

SAAD, A. N. et al. Incidence of oral clefts among different ethnicities in the state of california. **Annals of Plastic Surgery**, v. 72, n. SUPPL. 1, p. 81–83, 2014.

SALEEM, K. et al. Assessment of candidate genes and genetic heterogeneity in human non syndromic orofacial clefts specifically non syndromic cleft lip with or without palate. **Heliyon**, v. 5, n. 12, p. e03019, 2019.

SANDY, J. et al. Cleft lip and palate: Care configuration, national registration, and research strategies. **Journal of the World Federation of Orthodontists**, v. 9, n. January, p. S40–S44, 2020.

SATO, Y. et al. Population attributable fractions of modifiable risk factors for nonsyndromic orofacial clefts: A prospective cohort study from the Japan environment and children's study. **Journal of Epidemiology**, v. 31, n. 4, p. 272–279, 2021.

SHEHAN, J. N. et al. Cleft Palate in Newborns Diagnosed With Prematurity. **Otolaryngology - Head and Neck Surgery (United States)**, v. 165, n. 6, p. 887–894, 1 dez. 2021.

SHIBUKAWA, B. M. C. et al. Fatores associados à presença de fissura labial e/ou fenda palatina em recém-nascidos brasileiros. **Rev. Bras. Saúde Mater. Infant.**, v. 19, n. 4, p. 957–966, 2019.

SHRIDHAR, K. et al. Single nucleotide polymorphisms as markers of genetic susceptibility for oral potentially malignant disorders risk: Review of evidence to date. **Oral Oncology**, v. 61, p. 146–151, 2016.

SILVA, H. P. V. DA et al. Risk factors and comorbidities in Brazilian patients with orofacial clefts. **Brazilian oral research**, v. 32, p. e24, 2018.

SILVESTRIN, S. et al. Avaliação da incompletude da variável escolaridade materna nos registros das Declarações de Nascidos Vivos nas capitais brasileiras – 1996 a 2013. **Cadernos de saúde pública**, v. 33, n. 7, p. 1–3, 2017.

SIMON, L.; HASHMI, M.; BRAGG, B. Apgar Score. Em: **StatPearls Publishing**. FL: [s.n.].

SLAVEC, L. et al. Genetic markers for non-syndromic orofacial clefts in populations of European ancestry: a meta-analysis. **Scientific Reports**, v. 12, n. 1, p. 1–14, 2022.

SMILLIE, I. et al. Socioeconomic influence on orofacial cleft patient care. p. 1–5, 2015.

SOIM, A. et al. A population-based case–control study of the association between weather-related extreme heat events and orofacial clefts. **Birth Defects Research**, v. 110, n. 19, p. 1468–1477, 15 nov. 2018.

SOUSA, G. F. T. DE; RONCALLI, Â. G. Orofacial clefts in Brazil and surgical rehabilitation under the Brazilian National Health System. **Brazilian oral research**, v. 31, p. e23, 2017.

SPINA, V. et al. Classificação das fissuras lábio-palatinas. **Rev Hosp Clin Fac Med**, v. 27, n. 1, p. 5–6, 1972.

SPINDER, N. et al. Maternal occupational exposure and oral clefts in offspring. **Environmental Health: A Global Access Science Source**, v. 16, n. 1, p. 1–11, 2017.

SREEJITH, V. P. et al. Psychological effect of prenatal diagnosis of cleft lip and palate: A systematic review. **Contemporary Clinical Dentistry**, v. 9, n. 2, p. 304–308, 2018.

STEINWACHS, E. F. et al. Nonsyndromic cleft lip and palate is not associated with cancer or other birth defects. **American Journal of Medical Genetics**, v. 90, n. 1, p. 17–24, 2000.

STERNLICHT, M. D. et al. Prognostic value of PAI1 in invasive breast cancer: Evidence that tumor-specific factors are more important than genetic variation in regulating PAI1 expression. **Cancer Epidemiology Biomarkers and Prevention**, v. 15, n. 11, p. 2107–2114, 2006.

STOTHARD, K. J. et al. Maternal Overweight and Obesity and the Risk of Congenital Anomalies A Systematic Review and Meta-analysis. **JAMA**, v. 301, n. 6, p. 636–650, 2009.

STUPPIA, L. et al. Genetics of syndromic and nonsyndromic cleft lip and palate. **Journal of Craniofacial Surgery**, v. 22, n. 5, p. 1722–1726, 2011.

SUNG, S.; MAHDY, H. Cesarean Section. Em: **StatPearls Publishing**. FL: Treasure Island, 2023.

SZWARCWALD, C. L. et al. Avaliação das informações do Sistema de Informações sobre Nascidos Vivos (SINASC), Brasil. **Cadernos de Saúde Pública**, v. 35, n. 10, p. 1–13, 2019.

TAIOLI, E. et al. Cleft lip and palate in family members of cancer survivors. **NIH Public Access**, v. 28, n. 9, p. 958–962, 2010.

TEIXEIRA, J. L. A. **Distribuição, caracterização e determinação da incidência dos casos de fissura orofacial no Estado de Sergipe, Brasil**. [s.l.] Universidade Federal de Sergipe, 2015.

TOFFOLI, G.; CECCHIN, E. Clinical implications of genetic polymorphisms on stomach cancer drug therapy. **Pharmacogenomics Journal**, v. 7, n. 2, p. 76–80, 2007.

TRINDADE-SUEDAM, I. K. et al. Diabetes mellitus and drug abuse during pregnancy and the risk for orofacial clefts and related abnormalities. **Revista Latino-Americana de Enfermagem**, v. 24, n. 0, 2016.

UEKER, M. E. et al. Parenteral exposure to pesticides and occurence of congenital malformations: hospital-based case-control study. **BMC Pediatrics**, v. 16, n. 1, 2016.

VAN AALST, J. A.; KOLAPPA, K. K.; SADOVE, M. MOC-PSSM CME article: Nonsyndromic cleft palate. **Plastic and Reconstructive Surgery**, v. 121, n. 1S, p. 1–14, 2008.

VIEIRA, A. R. Association between the transforming growth factor alpha gene and nonsyndromic oral clefts: A HuGE review. **American Journal of Epidemiology**, v. 163, n. 9, p. 790–810, 2006.

VIEIRA, A. R. **Genetic Basis of Oral Health Conditions**. 1st. ed. [s.l.] Springer, 2021a.

VIEIRA, A. R. Cleft Lip/Palate and Cancer: a True Connection. **Revista Brasileira de Cancerologia**, v. 67, n. 4, p. 11–13, 2021b.

VIEIRA, A. R. A Need for Updating the Research Agenda for Cleft Lip and Palate and Extending the Cleft Team Model to Dental Medicine. **Frontiers in Dental Medicine**, v. 2, n. May, p. 1–5, 2021c.

VIEIRA, A. R. et al. Orofacial Cleft Frequency Differences Depending on Geographic Origin: Coast vs. Inland. **Frontiers in Dental Medicine**, v. 2, n. April, p. 1–6, 2021.

VIEIRA, A. R.; KHALIQ, S.; LACE, B. Risk of cancer in relatives of children born with isolated cleft lip and palate. **American Journal of Medical Genetics, Part A**, v. 158 A, n. 6, p. 1503–1504, 2012.

VIENA, C. S. et al. Epidemiological Study of Cleft Palate in the State of Bahia, Brazil. **Brazilian Journal of Medicine and Human Health**, v. 5, n. 4, p. 123–133, 2017.

VIJAYAN, V. et al. Association of WNT pathway genes with nonsyndromic cleft lip with or without cleft palate. **Cleft Palate-Craniofacial Journal**, v. 55, n. 3, p. 335–341, 2018.

VISSCHER, P. M.; HILL, W. G.; WRAY, N. R. Heritability in the genomics era - Concepts and misconceptions. **Nature Reviews Genetics**, v. 9, n. 4, p. 255–266, 2008.

VU, G. H. et al. Poverty and Risk of Cleft Lip and Palate: An Analysis of United States Birth Data. **Plastic and Reconstructive Surgery**, v. 149, n. 1, p. 169–182, 1 jan. 2022.

WALLER, D. K. et al. **Prepregnancy Obesity as a Risk Factor for Structural Birth DefectsArch Pediatr Adolesc Med**. [s.l: s.n.]. Disponível em: <http://archpedi.jamanetwork.com/>.

WANG, H. et al. Gene expression profiling analysis contributes to understanding the association between non-syndromic cleft lip and palate, and cancer. **Molecular Medicine Reports**, v. 13, n. 3, p. 2110–2116, 2016a.

WANG, Y. et al. The association of the CYP1A1 Ile462val polymorphism with head and neck cancer risk: Evidence based on a cumulative meta-analysis. **OncoTargets and Therapy**, v. 9, p. 2927–2934, 2016b.

WARNAKULASURIYA, S. Global epidemiology of oral and oropharyngeal cancer. **Oral Oncology**, v. 45, n. 4–5, p. 309–316, 2009.

WARNAKULASURIYA, S. et al. Oral potentially malignant disorders: A consensus report from an international seminar on nomenclature and classification, convened by the WHO Collaborating Centre for Oral Cancer. **Oral Diseases**, v. 27, n. 8, p. 1862–1880, 1 nov. 2021.

WETZEL, S. L.; WOLLENBERG, J. Oral Potentially Malignant Disorders. **Dental Clinics of North America**, v. 64, n. 1, p. 25–37, 2020.

WORLD HEALTH ORGANIZATION. **Addressing the Global Challenges of Craniofacial Anomalies: A Report of a WHO Meeting on International Research on Craniofacial Anomalies.** Genova: [s.n.].

WORLD HEALTH ORGANIZATION. **Preterm birth**. Disponível em: <https://www.who.int/news-room/fact-sheets/detail/preterm-birth>. Acesso em: 30 maio. 2023.

WORLD HEALTH ORGANIZATION (WHO). **International Statistical Classification of Diseases and Related Health Problems (ICD)**. Disponível em: <https://www.who.int/standards/classifications/classification-of-diseases>. Acesso em: 15 jan. 2022.

WORLEY, M. L.; PATEL, K. G.; KILPATRICK, L. A. Cleft Lip and Palate. **Clinics in Perinatology**, v. 45, n. 4, p. 661–678, 2018.

WYSZYNSKI, D. F. et al. Birth weight and gestational age of newborns with cleft lip with or without cleft palate and with isolated cleft palate. **The Journal of clinical pediatric dentistry**, v. 27, n. 2, p. 185–190, 2003.

WYSZYNSKI, D. F.; WU, M. H. S. Prenatal and Perinatal Factors Associated With Isolated Oral Clefting. **Cleft Palate-Craniofacial Journal**, v. 39, n. 33, 2002.

XU, D. P. et al. A Study on Environmental Factors for Nonsyndromic Cleft Lip and/or Palate. **Journal of Craniofacial Surgery**, v. 29, n. 2, p. 364–367, 2018.

YAZDY, M. M. et al. Priorities for future public health research in orofacial clefts. **Cleft Palate-Craniofacial Journal**, v. 44, n. 4, p. 351–357, 2007.

YILDIRIM, M. et al. Defining predictors of cleft lip and palate risk. **Journal of Dental Research**, v. 91, n. 6, p. 556–561, 2012.

YU, C.-C. et al. Hereditary Cleft Lip / Palate and Wilms Tumor : A Rare Association. **Cleft Palate Craniofac J.**, v. 39, n. 3, p. 376–9, 2001.

YU, Y. et al. Genome-wide analyses of non-syndromic cleft lip with palate identify 14 novel loci and genetic heterogeneity. **Nature Communications**, v. 8, n. 2, p. 1–11, 2017.

ZACK, M.; ADAMI, H.-O.; ERICSON, A. Maternal and Perinatal Risk Factors for Childhood Leukemia. **Cancer Research**, v. 51, p. 3696–3701, 1991.

ZAMBUZZI, M. R. W. **Estudo de associação de polimorfismos nos genes TCN2 e MTRR e fissura labiopalatina**. [s.l: s.n.].

ZHANG, Q. et al. A Bibliometric Analysis of Cleft Lip and Palate-Related Publication Trends From 2000 to 2017. **Cleft Palate-Craniofacial Journal**, v. 56, n. 5, p. 658–669, 1 maio 2019.

ZHANG, X. et al. Snail and Axin2 expression predict the malignant transformation of oral leukoplakia. **Oral Oncology**, v. 73, p. 48–55, 2017.

ZHANG, Z. et al. Wnt signalling in oral and maxillofacial diseases. **Cell Biology International**, v. 46, n. 1, 2022.

ZHAN, T.; RINDTORFF, N.; BOUTROS, M. Wnt signaling in cancer. **Oncogene**, v. 36, n. 11, p. 1461–1473, 2017.

ZHU, J. L. et al. Do parents of children with congenital malformations have a higher cancer risk? A nationwide study in Denmark. **British Journal of Cancer**, v. 87, n. 5, p. 524–528, 2002.

APÊNDICES

APÊNDICE I

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Universidade Estadual de Feira de Santana

Programa de Pós-Graduação em Saúde Coletiva

(Elaborado de acordo com a Resolução 466/2012-CNS/CONEP, Resolução 340/2004-CONEP/CNS/MS e Carta Circular 041/2015/CONEP/CNS/MS)

Você está sendo convidado(a) a participar da pesquisa **"ASSOCIAÇÃO DAS FISSURAS OROFACIAIS COM DESORDENS POTENCIALMENTE MALIGNAS E CÂNCER ORAL"** , desenvolvida pela professora Valéria Souza Freitas e equipe. Antes de decidir sobre sua participação é importante que entenda o porquê desse estudo e o que ele envolve.

O objetivo principal deste estudo é avaliar a relação entre fendas que ocorrem nos lábios e/ou no palato (céu da boca) com lesões que podem se tornar ou são câncer de boca e de garganta. Para isso, será realizada uma análise genética a fim estudar se genes relacionados ao aparecimento de lesões que podem se tornar ou são câncer de boca e de garganta são os mesmos que provocam fendas nos lábios e/ou no palato. A realização deste estudo ajudará a esclarecer como ocorrem estas doenças colaborando para a sua prevenção e tratamento. Estão sendo convidados para participar deste estudo pessoas que apresentam feridas, lesões brancas ou vermelhas na boca, além de pessoas que tem ou tiveram câncer de boca ou garganta, e ainda pessoas sem estas doenças.

Você pode escolher se participará ou não desta pesquisa. Você não é obrigado a colaborar com os pesquisadores se não quiser, nem receberá nenhuma remuneração por isso. Caso decida participar, mas mudar de ideia durante a pesquisa você poderá sair a qualquer momento sem nenhum dano, prejuízo ou constrangimento. Isto também não afetará em nada o seu atendimento na Clínica Odontológica da UEFS. Caso deseje colaborar, a sua participação consistirá em responder a perguntas de uma entrevista, realizar um exame e uma pequena raspagem na boca, coletar um pouco de saliva e permitir que sejam feitas fotografias do interior da boca. A entrevista com a duração aproximada de 20 minutos, será realizada em uma sala separada para evitar qualquer tipo de constrangimento durante as respostas as perguntas. O exame da boca é simples e não causa dor, mas você pode sentir um leve desconforto por permanecer com a boca aberta durante alguns minutos, o que será atentamente controlado, uma vez que se trata de um exame feito por profissional especializado, de posse do conhecimento necessário para a adequada condução do atendimento odontológico. Além disso, será realizada uma leve raspagem sobre a lesão e na bochecha, que também não causa dor, utilizando uma espátula de madeira. Você também deverá fornecer uma pequena quantidade de saliva (mais ou menos 4ml), deixando-a escorrer em um frasco coletor por 15 minutos. Essa saliva será congelada e os genes relacionados as lesões que podem se tornar ou são câncer de boca e de garganta serão extraídos por meio de uma analise feita em laboratório na Universidade de Pittsburgh que fica nos Estados Unidos. As fotografias para ajudar no acompanhamento e tratamento das lesões serão feitas apenas do interior da boca de modo a não permitir a sua identificação garantindo o seu anonimato.

Os seus dados e resultados dos exames genéticos não serão divulgados em nenhuma hipótese. Você tem a opção de escolher entre ser informado ou não sobre resultados de seus exames. Participando desta pesquisa, você estará contribuindo para a elaboração de um trabalho científico que poderá proporcionar benefícios futuros à sociedade. Os resultados dessa pesquisa podem também ajudar no desenvolvimento, pelos serviços públicos de saúde, de ações voltadas para prevenção e tratamento destas doenças; as quais trarão benefícios futuros para você, sua família e sua comunidade. Além disso, os participantes do estudo com lesões na boca permanecerão em acompanhamento periódico na clínica odontológica da UEFS para avaliação da evolução destas doenças, controle de eventuais sintomas e tratamento quando necessários.

Feira de Santana, __/_/_____

_____ _____

Assinatura OU Impressão datiloscópica do Participante da Pesquisa

Assinatura da Pesquisadora Principal
(Profª Valéria Souza Freitas)

São previstos riscos mínimos durante a sua participação nesse estudo, assim, caso venha a sentir qualquer constrangimento ou desconforto ao responder as perguntas da entrevista, durante o exame da boca e a coleta de material, tais procedimentos caso deseje podem ser interrompidos. Não é previsto que você tenha nenhuma despesa na participação nesta pesquisa, mas caso você venha a ter qualquer despesa em decorrência de sua contribuição neste estudo, você será plenamente ressarcido (Resolução CNS no 466 de 2012, itens II.21 e IV.3.g). Caso você sofra algum dano decorrente da sua participação nessa pesquisa, você tem o direito de buscar indenização (Resolução CNS no 466 de 2012, item IV.3.h) e ainda tem o direito à assistência integral e gratuita pelo tempo que for necessário (CONEP, Resolução CNS no 466 de 2012, itens II.3.1 e II.3.2).

Todas as informações coletadas sobre você durante a pesquisa serão mantidas em absoluto sigilo, não permitindo a sua identificação, assegurando a proteção de sua imagem e privacidade. Você pode a qualquer momento retirar sua autorização de uso e guarda da saliva coletada. Ao final da pesquisa, os materiais coletados serão descartados e todos os dados produzidos serão mantidos em arquivo sob a guarda da pesquisadora responsável, no Núcleo de Câncer Oral (NUCAO) da UEFS, por pelo menos 5 anos, conforme Resoluções do CNS nº 466/12 e nº 510/16 e, com o fim deste prazo, será descartado.

A divulgação dos resultados da pesquisa para os participantes da pesquisa será feita por meio de palestras previamente agendadas, como também para a comunidade da UEFS, bem como em congressos ou publicações científicas, mas a identidade dos participantes não será divulgada, nem serão utilizadas quaisquer imagens ou informações que permitam a sua identificação.

O desenvolvimento desta pesquisa seguirá rigorosamente todas as exigências preconizadas pela Resolução nº 466/12 - CNS/CONEP, Resolução nº340/2004 - CONEP/CNS/MS e Carta Circular 041/2015 - CONEP/CNS/MS. Em caso de dúvidas quanto aos seus direitos ou sobre o desenvolvimento deste estudo você pode a qualquer momento, durante a pesquisa, ou depois, entrar em contato com a pesquisadora responsável, Valéria Souza Freitas, com o Comitê de Ética em Pesquisa (CEP) da UEFS ou com a Comissão Nacional de Ética em Pesquisa (CONEP), seguem contatos abaixo. O CEP é um colegiado criado para defender os seus interesses como participante da pesquisa, garantindo a sia integridade e dignidade e para contribuir no desenvolvimento da pesquisa dentro de padrões éticos (Resolução CNS no 466 de 2012, item IV.5.d). O CONEP é uma comissão do Conselho Nacional de Saúde, com a função de implementar as normas e diretrizes regulamentadoras de pesquisas envolvendo seres humanos, aprovadas pelo Conselho.

Se quiser participar voluntariamente da pesquisa, autorizando a coleta, depósito, armazenamento e posterior análise da saliva na Universidade de Pittsburgh, bem como que os resultados da mesma sejam publicados, você e a pesquisadora responsável deverão assinar as duas páginas desse documento em duas vias: uma cópia ficará com você e a outra será recolhida pela pesquisadora responsável (Resolução CNS no 466 de 2012, item IV.5.d).

Contato da pesquisadora responsável:
Valéria Souza Freitas
Endereço: Centro de Pós-Graduação em Saúde Coletiva, Avenida Transnordestina, S/N, Bairro Novo Horizonte, Feira de Santa – Bahia, CEP: 44036-900
Telefone: (75) 3161- 8096
e-mail da pesquisadora: vfreitas@uefs.br

Contato do Comitê de Ética em Pesquisa da UEFS
Endereço: Universidade Estadual de Feira de Santana, Módulo 1, MA 17, Avenida Transnordestina, S/N, Bairro Novo Horizonte, Feira de Santa – Bahia, CEP: 44036-900
Telefone: (75) 3161-8124
Horário de Funcionamento: Segunda à Sexta, 13:30h – 17:30h
e-mail do Comitê: cep@uefs.br

Contato da Comissão Nacional de Ética em Pesquisa (CONEP)
Endereço: SRTV 701, Via W 5 Norte, lote D - Edifício PO 700, 3o andar – Asa Norte, Brasília – DF, CEP: 70719-040.
Telefone: (61) 3315-5878.
Horário de funcionamento: Segunda à Sexta, 09:00h as 18:00h
e-mail: **conep**.cep@saude.gov.br

Feira de Santana, __/_/_____

_____ _____
Assinatura OU Impressão datiloscópica do Participante da Pesquisa Assinatura da Pesquisadora Principal
(Profª Valéria Souza Freitas)

APÊNDICE II

FORMULÁRIO DE ENTREVISTA

PESQUISA: ASSOCIAÇÃO DAS FISSURAS OROFACIAIS COM DESORDENS POTENCIALMENTE MALIGNAS E CÂNCER ORAL

Data da coleta:__ / __ / 22 Nº do Participante: _____ Origem do participante: _____
Nome do pesquisador aplicante: _____
Tel.:_____

FATORES SOCIODEMOGRÁFICOS

1. Idade: _____ (anos)
2. Data de Nascimento: ___/___/_____
3. Cidade de origem: _____
4. Cidade de residência: _____
5. Sexo: ( ) Masculino ( ) Feminino
6. Etnia: ( ) Branca ( ) Preta ( ) Parda ( ) Amarelo ( ) Indígena
7. Escolaridade

( ) Fundamental ( ) Fundamental Incompleto ( ) Médio ( ) Médio Incompleto
( ) Superior ( ) Superior Incompleto ( ) Analfabeto

8. Qual a sua profissão? _____
9. Qual a sua ocupação no momento? _____
10. Estado civil: ( ) Solteiro ( ) Casado (União estável) ( ) Divorciado( ) Viúvo

DETERMINANTES BIOLÓGICOS

11. Tem algum grau de parentesco com seu cônjuge? ( )Sim ( ) Não
12. Se a resposta for positiva, qual o grau? _____
13. Tem filhos? ( )Sim ( ) Não 14. Se sim, quantos? _____

HISTÓRICO DE CÂNCER

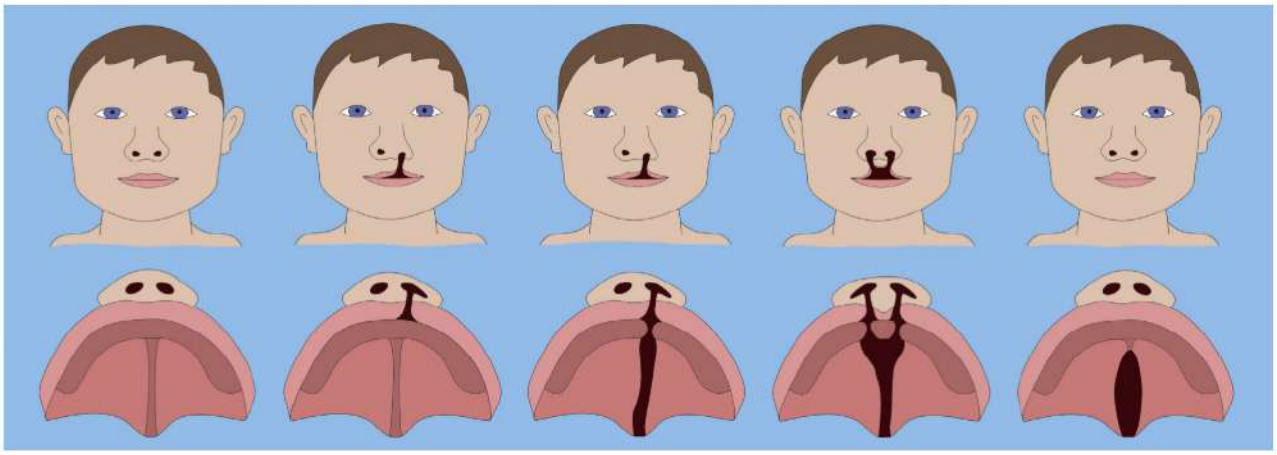
15. Alguém da sua família já teve câncer? ( )Sim ( ) Não
16. Se a resposta for positiva, qual o grau de parentesco? _____
17. Se a resposta for positiva, qual tipo?_____
18. Você já teve algum tipo de câncer? ( )Sim ( ) Não
19. Se a resposta for positiva, qual tipo? _____
20. Se a resposta for positiva, a quanto tempo? _____
21. Se a resposta for positiva , qual tratamento efetuado?

( ) Cirurgia ( ) Radioterapia ( ) Quimioterapia ( ) Combinado

HISTÓRICO DE AGENESIA DENTÁRIA

22. Você apresenta algum tipo de ausência de dentes? ( ) Sim ( ) Não
23. Se sim, lembra qual a causa da ausência de dentes? ( ) Exodontia, quais dentes? _____
( ) Agenesia, quais dentes? _____

HISTÓRICO DE FISSURAS OROFACIAIS



Normal Fissura de Lábio Fissura de Lábio e Palato Fissura de Palato

24. Você já teve algum tipo de fissura orofacial? ( ) Sim ( ) Não
25. Se sim, qual tipo? ( ) Fenda palatina ( ) Fenda labial ( ) Fenda labiopalatina
26. Realizou cirurgia reparadora? ( )Sim ( ) Não
27. Há quanto tempo? _____
28. Tem algum caso na família com fissura orofacial? ( )Sim ( ) Não
29. Se a resposta for positiva, qual o grau de parentesco? _____

HÁBITOS DE VIDA E COMPORTAMENTAIS

30. Sua mãe fumou durante a sua gestação? ( )Sim ( ) Não
31. Seu pai fumava quando sua mãe estava grávida? ( )Sim ( ) Não
32. Quantos anos sua mãe tinha ao você nascer? _____
33. Quantos anos seu pai tinha ao você nascer? _____

34. Você fuma? ( )Sim ( ) Não
35. Se a resposta for positiva, qual o tipo? _____
36. Se a resposta for positiva, há quanto tempo você fuma? _____
37. Você já fumou? ( )Sim ( ) Não
38. Se a resposta for positiva, qual o tipo? _____
39. Se a resposta for positiva, há quanto tempo você parou de fumar? _____

40. Você consome bebidas alcoólicas? ( )Sim ( ) Não
41. Se a resposta for positiva, qual tipo de bebida? _____
42. Você já consumiu bebidas alcoólicas? ( )Sim ( ) Não
43. Se a resposta for positiva, qual o tipo? _____
44. Se a resposta for positiva, há quanto tempo você parou de consumir bebidas alcoólicas? _____

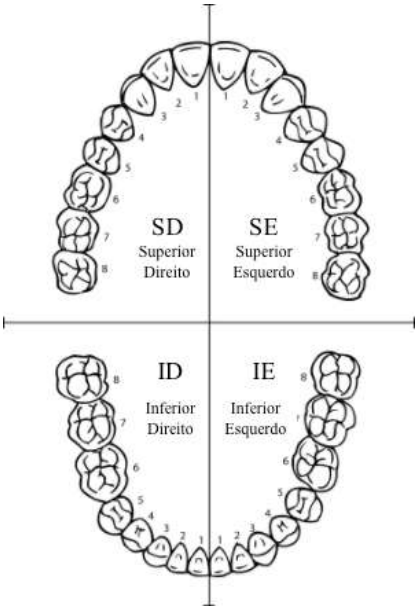
APÊNDICE III

FORMULÁRIO DE EXAME CLÍNICO

PESQUISA: ASSOCIAÇÃO DAS FISSURAS OROFACIAIS COM DESORDENS POTENCIALMENTE MALIGNAS E CÂNCER ORAL

Data da coleta:__ / __ / 22 Nº do Participante: _____ Origem do participante: _____
Nome do pesquisador aplicante: _____ Tel.: _____

UNIDADES DENTÁRIAS AUSÊNTES

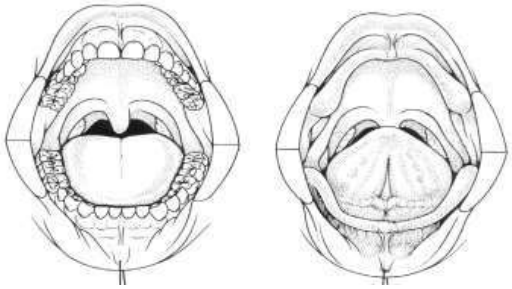


Unidade ausente	Causa da ausência	
	Exodontia	Agenesia

DADOS SOBRE A LESÃO POTENCIALMENTE MALIGNA OU DE CÂNCER ORAL

45. Participante apresenta diagnostico de lesão potencialmente maligna ou de câncer oral? ( )Sim ( ) Não
46. Em caso positivo, qual? _____

Marcar região anatômica da lesão



47. **Informações Adicionais** (coletadas do portuário clínico)
48. Tipo histológico: _____
49. Localização anatômica: _____
50. Estadiamento: _____
51. Tratamento efetuado: ( ) Cirurgia ( ) Radioterapia ( ) Quimioterapia ( ) Combinado
52. Qual a combinação do tratamento: _____
53. Estágio de tratamento: _____

ANEXOS

UNIVERSIDADE ESTADUAL DE FEIRA DE SANTANA - UEFS

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: ASSOCIAÇÃO DAS FISSURAS OROFACIAIS COM DESORDENS POTENCIALMENTE MALIGNAS E CÂNCER ORAL

Pesquisador: Valéria Souza Freitas

Área Temática: Genética Humana:
(Haverá envio para o exterior de material genético ou qualquer material biológico humano para obtenção de material genético, salvo nos casos em que houver cooperação com o Governo Brasileiro;);

Versão: 2

CAAE: 61386322.8.0000.0053

Instituição Proponente: Universidade Estadual de Feira de Santana

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 5.820.219

Apresentação do Projeto:

As Fissuras Orofaciais (FO) e o câncer oral são problemas de saúde pública que podem ocasionar alterações estéticas, funcionais e emocionais(AGBENORKU, 2013; ASSIS et al., 2020; FARIA, 2019; GUPTA; JOHNSON; KUMAR, 2016; MACHADO et al., 2018; MALICKA; HOJAN; HANUSZKIEWICZ, 2021; MURRAY, 2002; SONG et al., 2018), contribuindo para uma maior morbidade e mortalidade em comparação com a população geral(MOSSEY et al., 2009; SUNG et al., 2021). Sua etiologia é complexa e multifatorial, envolvendo fatores genéticos e ambientais, que podem atuar isoladamente ou em associação(DIXON et al., 2011; JOHNSON et al., 2020; LESLIE; MURRAY, 2013; MOSSEY et al., 2009).A expressão de alguns genes como IRF6, 8MTHFR, GRHL3, VAX1, TGFA e TGFB3, FOXE1, NOG, GREM1, AXIN2, DVL2, WNT3A e WNT5A, SUMO1, MSX1, TBX22, PAX7, MAFB, CYP1A1, GSTM1 e NAT2 foi associada ao aumento do risco para o desenvolvimento de FO(KHAN; PRASHANTH; SRINATH, 2020; LAMMER et al., 2004; LESLIE; MARAZITA, 2013; MACHADO et al., 2018; MENEZES et al., 2009; SALEEM et al., 2019; SLAVEC et al., 2022; STUPPIA et al., 2011; VIEIRA, 2006, 2021a). Por outro lado, este processo muitas vezes pode não ser atribuído a uma única mutação genética facilmente identificável, mas a uma complexa interação entre fatores genéticos e exposições ambientais, com variações raciais, geográficas e socioeconômicas(DIXON et al., 2011; LESLIE; MARAZITA, 2013; MOSSEY et al., 2009).Fatores ambientais podem influenciar a

Endereço: Avenida Transnordestina, s/n - Novo Horizonte, UEFS
Bairro: Módulo I, MA 17 **CEP:** 44.031-460
UF: BA **Município:** FEIRA DE SANTANA
Telefone: (75)3161-8124 **E-mail:** cep@uefs.br

expressão gênica a partir de mudanças epigenéticas (NOTTERMAN; MITCHELL, 2015). As inequidades socioeconômicas, desigualdades nas condições de vida e trabalho e aspectos sociais relacionados a um baixo nível de coesão social geram uma maior susceptibilidade a exposição de fatores ambientais e a um estresse crônico que podem influenciar a situação de saúde por meio de diversos mecanismos biológicos(NOTTERMAN; MITCHELL, 2015). Fatores ambientais e de estilo de vida foram associados ao desenvolvimento das FO, como a situação socioeconômica, etnia, idade dos pais, peso ao nascer, tabagismo, consumo de bebidas alcoólicas, ingestão de drogas/medicamentos, região geográfica, poluição, ocupação, doenças da mãe, exposições a herbicidas/pesticidas e à radiação ionizante(GARLAND et al., 2020; KAPOS et al., 2021; NASREDDINE; EL HAJJ; GHASSIBE-SABBAGH, 2021; SAAD et al., 2014; VIEIRA, 2021b). Fatores como o tabagismo e o consumo abusivo de bebidas alcóolicas também estão relacionados ao câncer oral, podendo contribuir individualmente ou atuar sinergicamente para promover o processo de carcinogênese (MCMAHON; CHEN, 2003). Outros fatores como a exposição a poluentes ambientais(SANKPAL et al., 2012) e infecção por agentes virais como o Papilomavírus Humano (HPV) também foram associados a etiologia do câncer de cavidade oral e orofaringe(JOHNSON et al., 2020). Genes reguladores do metabolismo de substâncias carcinogênicas como o tabaco e álcool, podem estar envolvidos no processo de carcinogênese, mas considerando que nem todos os indivíduos expostos a estes agentes agressores desenvolvem o câncer oral, o risco para o desenvolvimento da doença pode estar relacionado à suscetibilidade individual, modulada por polimorfismos genéticos. Dois ou mais fenótipos resultantes de variações alélicas de ocorrência simultânea nos genes que codificam as enzimas envolvidas no metabolismo do tabaco e do álcool, e em genes de reparo do DNA, assim como, a interação entre eles, podem alterar o resultado final da exposição e, portanto, tornarem-se um importante fator do hospedeiro, favorável ou não ao desenvolvimento do câncer(CABRAL et al., 2010; DATTA et al., 2007; GATTAS et al., 2014).

Objetivo da Pesquisa:

Objetivo Primário:

Avaliar a associação entre as fissuras orofaciais não sindrômicas e desordens potencialmente malignas e câncer oral.

Objetivos Secundários:

- Determinar a frequência de casos de fissuras orofaciais não sindrômicas em indivíduos com desordens potencialmente malignas e câncer oral;

- Determinar a frequência de casos de fissuras orofaciais não sindrômicas em parentes de primeiro grau indivíduos com desordens potencialmente malignas e câncer oral;
- Determinar a frequência de casos de agenesia dentária em indivíduos com desordens potencialmente malignas e câncer oral;
- Descrever o perfil clínico e fatores socioeconômicos, hábitos comportamentais e de estilo de vida em indivíduos com desordens potencialmente malignas e câncer oral;
- Avaliar a associação de fatores ambientais relacionados as fissuras orofaciais com desordens potencialmente malignas e câncer oral;
- Avaliar a associação entre histórico familiar de fissuras orofaciais não sindrômicas em parentes de primeiro grau e a ocorrência de desordens potencialmente malignas e câncer oral;
- Analisar a presença de polimorfismos genéticos relacionados a fissuras orofaciais não sindrômicas em indivíduos com desordens potencialmente malignas e câncer oral;
- Avaliar a associação entre fatores socioeconômicos, hábitos comportamentais e de estilo de vida com polimorfismos genéticos em indivíduos com desordens potencialmente malignas e câncer oral.

Avaliação dos Riscos e Benefícios:

Riscos:

Os indivíduos serão esclarecidos antes e durante a realização da pesquisa tendo a liberdade de recusar ou desistir de participar da pesquisa em qualquer fase, sem penalização alguma e sem prejuízo ao seu cuidado. A entrevista será realizada em local reservado, respeitando a privacidade do participante, buscando reduzir riscos ou danos ao mesmo. O sujeito será esclarecido que caso alguma pergunta cause desconforto ou constrangimento ele poderá se recusar a responder. Os sujeitos da pesquisa serão submetidos ao exame da cavidade bucal, a coleta de saliva, a coleta de esfregaço da mucosa e a fotografias. O exame bucal é muito simples, não causando dor e riscos como lesões nos tecidos moles. Leve desconforto de permanecer de boca aberta durante alguns minutos serão atentamente controlados, uma vez

que se trata de um exame feito por profissional especializado, de posse do conhecimento necessário para a adequada condução do atendimento odontológico. As fotografias serão realizadas apenas com a anuência dos sujeitos da pesquisa e exclusivamente das lesões do interior da boca, não permitindo a identificação da face dos sujeitos da pesquisa, mantendo o seu anonimato. As fotografias serão realizadas de modo a permitir o acompanhamento da evolução das lesões para o estabelecimento de protocolos mais adequados de tratamento. A coleta de saliva, bem como do esfregaço da mucosa, são métodos não invasivos, minimizando riscos de

contaminação e desconforto conforme descrito na coleta de dados. De forma a
garantir a confidencialidade, anonimato e privacidade dos dados coletados estes serão armazenados em um armário com chave em uma sala de acesso restrito do Núcleo de Câncer Oral (NUCAO). Além disso, todas as versões eletrônicas dos dados coletados serão mantidas em arquivos protegidos com senha, mantidos em computadores também de acesso restrito do NUCAO. Ao final da pesquisa, todo material será mantido em arquivo sob a guarda da pesquisadora responsável, no Núcleo de Câncer Oral (NUCAO) da UEFS, por pelo menos 5 anos, conforme Resoluções do CNS nº 466/12 e nº 510/16 e, com o fim deste prazo, será descartado (BRASIL, 2012).

Benefícios:
A identificação de fatores genéticos e ambientais relacionados ao risco de FO em pacientes com desordens orais potencialmente malignas e câncer oral poderá permitir o desenvolvimento de estratégias e uma abordagem preventiva na identificação de grupos de risco, prevenção e controle destas doenças. Os resultados do presente estudo podem também contribuir para a realização de estudos populacionais que investiguem a associação entre FO e câncer oral, a fim de esclarecer essa relação, permitindo o desenvolvimento de políticas públicas. Além disso, os indivíduos com estas doenças participantes do estudo permanecerão em acompanhamento periódico na clínica odontológica da UEFS para controle de eventuais sintomas e da evolução destas doenças. A divulgação dos resultados do projeto de pesquisa será realizada para os participantes da pesquisa, instituição e para a sociedade através de publicações e apresentações em eventos científicos mantendo o anonimato dos sujeitos da pesquisa.

Comentários e Considerações sobre a Pesquisa:
A pesquisa tem importância clínica e epidemiológica para a pesquisa científica envolvendo parâmetros moleculares de marcação genética para a identificação e diagnóstico de câncer orofacial como demonstrado na redação do projeto.

Considerações sobre os Termos de apresentação obrigatória:
No Apêndice II do arquivo Projeto_CEP_Final.pdf, Formulário de Entrevista, é solicitado o nome do paciente, ou seja , o participante vai ser identificado, o que não está de acordo com a orientação das resoluções do CNS sobre o direito à privacidade do participante, devendo portanto ser revista essa solicitação de identificação.
Esta pesquisa irá trabalhar com DNA humano extraído dos participantes e nesse caso as declarações de anuência deverão ter explicitamente declarado, por parte da pesquisadora

responsável e demais integrantes da equipe de pesquisa que irão obedecer as normas contidas na RESOLUÇÃO Nº 304 DE 09 DE AGOSTO DE 2000 e também à Carta Circular nº 041/2015/CONEP/CNS/MS, que explica o Item V.1.a da Resolução CNS 304/2015. Na metodologia não há explicação de como será garantida a privacidade do atendimento ao participante da pesquisa, já que para a entrevista a privacidade está garantida pela pesquisa.

Conclusões ou Pendências e Lista de Inadequações:

Trata-se de apreciação de retorno de pendência:

Pendência

1) Corrigir o termo sujeito de pesquisa por participantes da pesquisa conforme as resoluções CNS 466/2012 e 510/2016

PENDÊNCIA ATENDIDA

2) Esclarecer quem arcará com os custos dos exames laboratoriais

Os custos dos exames laboratoriais serão de responsabilidade dos próprios pesquisadores, sem ónus aos participantes da pesquisa, conforme descrito no item Orçamento do Projeto Detalhado (página 34).

PENDÊNCIA ATENDIDA

3) Rever a solicitação da identificação do paciente no formulário apresentado no Apêndice II do Projeto de Pesquisa.

A solicitação de identificação do participante foi removida dos Apêndices II (FORMULÁRIO DE ENTREVISTA) e III (FORMULÁRIO DE EXAME CLÍNICO), páginas 54 e 56, respectivamente, garantindo o anonimato dos participantes da pesquisa.

PENDÊNCIA ATENDIDA

4) Explicar como será realizada a privacidade do atendimento ao paciente

Para assegurar a privacidade e evitar qualquer tipo ou situação de estigmatização e discriminação dos participantes da pesquisa, da família e do grupo, bem como reduzir riscos ou danos ao mesmo, a entrevista será realizada em uma sala separada do ambiente clínico, onde estará no momento da pesquisa somente o próprio participante e o pesquisador aplicante", adicionado no item Possíveisriscos/desconfortos, página 31, do Projeto Detalhado. Os participantes da pesquisa

serão submetidos ao exame da cavidade bucal, a coleta de saliva, a coleta de esfregaço da mucosa e a fotografias. Para garantir a privacidade do participante durante essa etapa, o participante será atendido em um box de atendimento clínico odontológico individual, presente no momento da coleta apenas o participante e o pesquisador aplicante" – adicionado ao item Possíveis riscos/desconfortos, página 31, do Projeto Detalhado.
PENDÊNCIA ATENDIDA

5) Refazer as redações de todas das declarações de anuência incluindo que as/os integrante da equipe irão obedecer, também, a Resolução CNS 340/2004 e a Carta Circular 041/2015/CONEP/CNS/MS.
As declarações de anuência da pesquisadora responsável e demais integrantes da equipe de pesquisa foram revisadas a fim de incluir as solicitações da RESOLUÇÃO no 340/ 2004 (Diretrizes para Análise Ética e Tramitação dos Projetos de Pesquisa da Área Temática Especial de Genética Humana) e também da Carta Circular no 041/2015/CONEP/CNS/MS.
PENDÊNCIA ATENDIDA

6) Refazer a redação do TCLE observando as normas determinadas e solicitadas na Resolução 340/2004 e também na Carta Circular no 041/2015/CONEP/CNS/MS, que explica o Item V.1.a da Resolução CNS 340/2004.
A redação do TCLE foi revisada a fim de incluir as recomendações da RESOLUÇÃO no 340/ 2004 (Diretrizes para Análise Ética e Tramitação dos Projetos de Pesquisa da Área Temática Especial de Genética Humana) e também da Carta Circular no 041/2015/CONEP/CNS/MS.
PENDÊNCIA ATENDIDA

7) Entende-se que a terminologia adotada pela Resolução CNS no 466 de 2012, item II.10 – participante de pesquisa deva ser empregada em todos os documentos do protocolo de pesquisa, incluindo o TCLE, em substituição a voluntário, paciente, etc. Solicita-se adequação.
Alteração do termo "sujeitos da pesquisa" por "participantes da pesquisa" nas páginas 2, 4, 31, 32, 34, 35 do Projeto Detalhado; Alteração do termo "indivíduos" por "participante" na sessão Possíveis riscos/desconfortos do Projeto Detalhado (página 31); Alteração do termo "pacientes"

por “participantes” na sessão Possíveis riscos/desconfortos do Projeto Detalhado (página 31); Alteração do termo “paciente” por “participante” no Apêndice II do Projeto
Detalhado (página 55); Alteração do termo “paciente” por “participante” no Apêndice III) do Projeto Detalhado (página 56); Alteração do termo “paciente” por “participante” na página 1 do Formulário de Entrevista; Alteração do termo “paciente” por “participante” na página 1 do Formulário de Exame Clínico.
PENDÊNCIA ATENDIDA

8) Documentação
• As resoluções e cartas mencionadas no parecer foram incluídas às declarações
dos(as) pesquisadores(as) envolvidos(as).
PENDÊNCIA ATENDIDA

Após o atendimento das pendências, a EMENDA proposta está aprovada para execução, pois atende aos princípios bioéticos para pesquisa envolvendo seres humanos, conforme a Resolução nº 466/12 (CNS).

Considerações Finais a critério do CEP:
Tenho muita satisfação em informar-lhe que seu Projeto de Pesquisa satisfaz às exigências da Res. 466/12 e 510/2016 e da norma operacional 001/2013. Assim, seu projeto foi Aprovado, podendo ser iniciada a coleta de dados com os participantes da pesquisa conforme orienta o Cap. X.3, alínea a - Res. 466/12 e Cap II da Res 510/2016. Relembro que conforme institui a Res. 466/12 e 510/2016, Vossa Senhoria deverá enviar a este CEP relatórios anuais de atividades pertinentes ao referido projeto e um relatório final tão logo a pesquisa seja concluída. Em nome dos membros CEP/UEFS, desejo-lhe pleno sucesso no desenvolvimento dos trabalhos e, em tempo oportuno, um ano, este CEP aguardará o recebimento dos referidos relatórios.

O presente projeto, seguiu nesta data para análise da CONEP e só tem o seu início autorizado após a aprovação pela mesma.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Continuação do Parecer: 5.820.219

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1994233.pdf	19/10/2022 09:02:50		Aceito
Folha de Rosto	FolhaDeRosto_assinada_nova.PDF	19/10/2022 08:57:35	Valéria Souza Freitas	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_CEP_Final_corrigido.pdf	19/10/2022 06:58:40	Valéria Souza Freitas	Aceito
Declaração de Pesquisadores	Anuencia_Valeria_alterado.pdf	19/10/2022 06:53:22	Valéria Souza Freitas	Aceito
Recurso Anexado pelo Pesquisador	CARTA_Resposta_PENDENCIAS_PARECER.pdf	19/10/2022 06:49:56	Valéria Souza Freitas	Aceito
Outros	Termo_confidencialidade.pdf	19/10/2022 06:44:24	Valéria Souza Freitas	Aceito
Outros	Dec_publicacao_resultados.pdf	19/10/2022 06:44:01	Valéria Souza Freitas	Aceito
Outros	Dec_destinacao_material.pdf	19/10/2022 06:43:23	Valéria Souza Freitas	Aceito
Outros	Formulario_entrevista_corrigido.pdf	19/10/2022 06:41:03	Valéria Souza Freitas	Aceito
Outros	Formulario_exame_clinico_corrigido.pdf	19/10/2022 06:36:39	Valéria Souza Freitas	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_corrigido.pdf	19/10/2022 06:36:06	Valéria Souza Freitas	Aceito
Declaração de Pesquisadores	Anuencia_Ynara_alterado.pdf	19/10/2022 06:33:28	Valéria Souza Freitas	Aceito
Declaração de Pesquisadores	Anuencia_Michelle_alterado.pdf	19/10/2022 06:33:14	Valéria Souza Freitas	Aceito
Declaração de Pesquisadores	Anuencia_Clara_alterado.pdf	19/10/2022 06:32:59	Valéria Souza Freitas	Aceito
Declaração de Pesquisadores	Anuencia_Angela_alterado.pdf	19/10/2022 06:32:41	Valéria Souza Freitas	Aceito
Declaração de Pesquisadores	Anuencia_Ana_Paula_alterado.pdf	19/10/2022 06:32:25	Valéria Souza Freitas	Aceito
Declaração de Pesquisadores	Anuencia_Alexandre_alterado.pdf	19/10/2022 06:31:56	Valéria Souza Freitas	Aceito
Declaração de Pesquisadores	Anuencia_Alesandra_alterado.pdf	19/10/2022 06:31:37	Valéria Souza Freitas	Aceito
Declaração de Pesquisadores	Anuencia_Adriana_alterado.pdf	19/10/2022 06:30:40	Valéria Souza Freitas	Aceito
Declaração de Manuseio Material Biológico / Biorepositório / Biobanco	Dec_manuseio_material.pdf	19/10/2022 06:25:39	Valéria Souza Freitas	Aceito

Endereço: Avenida Transnordestina, s/n - Novo Horizonte, UEFS
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Cronograma	Cronograma_alterado.pdf	19/10/2022 06:24:16	Valéria Souza Freitas	Aceito
Declaração de Instituição e Infraestrutura	Carta_Anuencia_NUCAO_assinada.pdf	05/08/2022 20:31:37	Valéria Souza Freitas	Aceito
Declaração de Instituição e Infraestrutura	Carta_anuencia_DSAU_assinada.pdf	05/08/2022 18:25:38	Valéria Souza Freitas	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_CEP_Final.pdf	05/08/2022 15:00:05	Valéria Souza Freitas	Aceito
Cronograma	Cronograma_atividades_CEP.pdf	05/08/2022 14:29:23	Valéria Souza Freitas	Aceito
Outros	Formulario_exame_clinico.pdf	05/08/2022 13:26:04	Valéria Souza Freitas	Aceito
Orçamento	Orcamento.pdf	05/08/2022 13:02:03	Valéria Souza Freitas	Aceito
Declaração de Pesquisadores	Anuencia_Ynara.pdf	05/08/2022 12:48:04	Valéria Souza Freitas	Aceito
Declaração de Pesquisadores	Anuencia_Michelle.pdf	05/08/2022 12:47:51	Valéria Souza Freitas	Aceito
Declaração de Pesquisadores	Anuencia_Clara.pdf	05/08/2022 12:47:27	Valéria Souza Freitas	Aceito
Declaração de Pesquisadores	Anuencia_Angela.pdf	05/08/2022 12:47:10	Valéria Souza Freitas	Aceito
Declaração de Pesquisadores	Anuencia_Ana_Paula.pdf	05/08/2022 12:46:51	Valéria Souza Freitas	Aceito
Declaração de Pesquisadores	Anuencia_Alesandra.pdf	05/08/2022 12:45:45	Valéria Souza Freitas	Aceito
Declaração de Pesquisadores	Anuencia_Alexandre.pdf	05/08/2022 12:45:23	Valéria Souza Freitas	Aceito
Declaração de Pesquisadores	Anuencia_Adriana.pdf	05/08/2022 12:43:04	Valéria Souza Freitas	Aceito
Declaração de Pesquisadores	Anuencia_Valeria.pdf	05/08/2022 12:39:37	Valéria Souza Freitas	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.pdf	05/08/2022 12:38:07	Valéria Souza Freitas	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Sim

Continuação do Parecer: 5.820.219

FEIRA DE SANTANA, 15 de Dezembro de 2022

Assinado por:
Wânia Silveira da Rocha
(Coordenador(a))

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