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ASSOCIAÇÃO ENTRE A HIPOMINERALIZAÇÃO DE MOLAR-INCISIVO, ASMA E BACTÉRIAS CARIOGÊNICAS

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“Conhecimento não é aquilo que você sabe, mas o que você faz com aquilo que você sabe”

- Aldous Huxley

RESUMO

As doenças respiratórias infantis, como a asma, são importantes problemas de saúde pública em todo o mundo e podem estar associadas a defeitos no esmalte dentário. Dentes com defeitos significativos em sua estrutura, como os acometidos pela Hipomineralização de Molar-Incisivo (HMI), são mais susceptíveis ao acúmulo de biofilme e às lesões cariosas. A cárie é uma doença multifatorial de significativa prevalência na infância e a presença do biofilme é um fator crucial para seu desenvolvimento. Assim, pesquisas que abordem HMI e cárie conjuntamente devem ser estimuladas, visto que o controle dessas condições permanece um desafio na atualidade. Esta tese, constituída por 2 capítulos teve como objetivos: 1. Revisar sistematicamente artigos sobre a associação entre asma e defeitos de esmalte em dentes decíduos e permanentes jovens (recém-erupcionados); 2. Avaliar por meio de um estudo transverso a relação entre os estágios de HMI e a presença de bactérias cariogênicas em crianças. No levantamento dos artigos da revisão (2000-2021) foram incluídas quatro bases de dados (PUBMED-MEDLINE, EMBASE, LILACS, COCHRANE). Os dados da revisão foram avaliados qualitativamente. Vinte e dois artigos foram criticamente avaliados, e a relação da asma e defeitos de esmalte foi encontrada em 1 de alto nível de evidência, 9 com nível moderado e 4 com nível baixo. Por meio da revisão conclui-se que asma está intimamente associada a defeitos de esmalte em dentes permanentes jovens. No estudo transverso, 566 escolares (6-8 anos), de Bragança Paulista-SP, foram submetidos a avaliação de presença de HMI e cárie, por meio de exames clínicos utilizando os critérios da Associação Europeia de Odontopediatria para HMI e o critério da Organização Mundial da Saúde modificado para cárie. Quarenta escolares foram distribuídos igualmente em 4 grupos: G1-Primeiros molares permanentes saudáveis; G2 - HMI leve com opacidade branca e livre de cárie, G3 - HMI leve com opacidade amarela e livre de cárie, G4 - HMI grave com opacidade branca/amarela e presença de cárie. O biofilme dental foi avaliado para quantificação de *Streptococcus mutans* e *Lactobacillus* spp. por PCR em tempo real, e a ingestão de açúcar pelo Questionário Alimentar do Dia Anterior. Os dados do estudo transverso foram analisados por análise de variância seguida do teste de Tukey, Qui-quadrado e regressão de Poisson. As crianças sem HMI e sem cárie (G1) e as com HMI leve (G2) comportam-se de forma semelhante quando comparadas com G3 e G4. Além disso, o teste de Tukey evidenciou que HMI grave juntamente com cárie (G4) apresentou maior proporção de *S. mutans* e *Lactobacillus* spp. quando comparado com G2 ($p<0,05$). Curiosamente, aumentos nos níveis de *Lactobacillus* spp. foram associados a um aumento da severidade da HMI (RR:7; $p=0,03$). As conclusões do estudo transversal revelaram que os níveis de *Lactobacillus* spp. foram influenciados por diferentes graus de HMI e a presença de cárie. É importante salientar que identificar HMI precocemente, como logo após a erupção desses elementos na cavidade bucal, conforme realizado no presente estudo, possibilita pontuar quem está sob risco, favorecendo o direcionamento efetivo dos tratamentos preventivos.

***Palavras-chave**: Cárie dentária. Suscetibilidade à Cárie Dentária. Desmineralização do Dente. Hipomineralização dentária.*

ABSTRACT

Childhood respiratory diseases, such as asthma, are important public health problems worldwide and may be associated with defects in tooth enamel. Teeth with significant defects in their structure, such as those affected by molar-incisor hypomineralization (MIH), are more susceptible to biofilm accumulation and carious lesions. Caries is a multifactorial disease with high prevalence in childhood, and the presence of biofilm is a critical factor in its development. Therefore, research addressing MIH and caries together should be encouraged, as the control of these conditions is a challenge nowadays. This thesis, consisting of 2 chapters, aimed to: 1. Systematically review papers about the relationship between asthma and enamel defects in deciduous and young permanent teeth (early-erupted). 2. To evaluate with a transverse study the relationship between stages of MIH and the presence of cariogenic bacteria in children. Four databases were included (PUBMED-MEDLINE, EMBASE, LILACS, COCHRANE) in the literature search of the review (2000-2021). Twenty-two articles were critically evaluated, and the relationship between asthma and enamel deffects was found to be 1 with a high level of evidence, 9 with a moderate level, and 4 with a low level. The review concludes that asthma is closely connected with enamel defects in young permanent teeth. In the cross-sectional study, 566 schoolchildren (6-8 years) from Bragança Paulista (SP) were subjected to an assessment of MIH and caries, by clinical examination using the European Association of Pediatric Dentistry criteria for MIH and the modified World Health Organization criteria for caries. Forty students were divided into 4 groups: G1- First permanent molars; G2 - Mild MIH with white opacity and caries-free, G3 - Mild MIH with yellow opacity and caries-free, G4 - Severe MIH with white/yellow opacity and presence of caries. Dental biofilm was assessed for quantification of *Streptococcus mutans* and *Lactobacillus* spp. using real-time PCR, and sugar intake was assessed using the Previous Day Food Questionnaire. Data from the review were analyzed qualitatively, whereas data from the cross-sectional study were evaluated using analysis of variance followed by Tukey test, Chi-square, and Poisson regression. Children without MIH and caries (G1) and those with mild MIH (G2) behaved similarly compared with G3 and G4. In addition, Tukey's test showed that severe MIH along with caries (G4) had a higher proportion of *S. mutans* and *Lactobacillus* spp. compared to G2 ($p < 0.05$). Interestingly, an increase in levels of *Lactobacillus* spp. was associated with an increase in the severity of MIH (RR:7; p=0.03). The conclusions of the cross-sectional study revealed that the levels of *Lactobacillus* spp. were influenced by different degrees of MIH and caries. It is important to point out that the identification of MIH at an early stage, shortly after tooth eruption in the oral cavity, as in the present study, makes it possible to identify the ones at risk, which favors the targeting of effective preventive measures.

Keywords*: Dental caries. Susceptibility to Dental Caries. Tooth Demineralization. Tooth hypomineralization.*

LISTA DE SÍMBOLOS E ABREVIAÇÕES

HMI – Hipomineralização de molar-incisivo

LISTA DE FIGURAS

SUMÁRIO

1. Introdução

A Hipomineralização de molar-incisivo (HMI), pode ser evidenciada em crianças no início da dentição mista, pode acometer um ou vários dentes, com uma ou mais faces envolvidas, fatores relacionados a saúde geral como: complicações pré, peri e pós-natais, uso frequente de antibióticos, doenças de primeira infância, asma e polimorfismo genético parecem estar associados à sua presença (DE OLIVEIRA RIBAS; CZLUSNIAK, 2004, WHATLING, 2008, KÜHNISCH et al., 2016, BEZAMAT, 2021).

Os defeitos de esmalte podem ser dos tipos qualitativos (hipomineralização, observando-se alterações de cores, mas mantendo-se o volume e tamanho do dente) e quantitativos (hipoplasia, onde ocorre uma perda de volume, observando-se uma falta de estrutura do esmalte) (WEERHEIJM et al., 2004). A HMI é um defeito de origem sistêmica, que ocorre no esmalte dentário obrigatoriamente de molares permanentes, podendo ou não acometer os incisivos permanentes (FIGURA 1). Também pode ser encontrado em segundos molares e incisivos decíduos sendo denominados de hipomineralização de molares decíduos (FAGRELL et al., 2010). Um artigo recente sugeriu que a nomenclatura HMI deveria ser substituída por Hipomineralização Molar simplificando a linguagem usada para descrever esse distúrbio onde o fenótipo atualmente aceito inclui uma opacidade demarcada em qualquer molar ± qualquer outro dente, de dentição decídua ou permanente, sendo importante para a padronização da terminologia comum (COOK; LOPEZ, 2022). É um tema bastante discutido na atualidade, sendo metade dos artigos na base de dados PUBMED pesquisados com as palavras chave: “Molar incisor hypomineralisation” e “child” publicados nos últimos 5 anos.

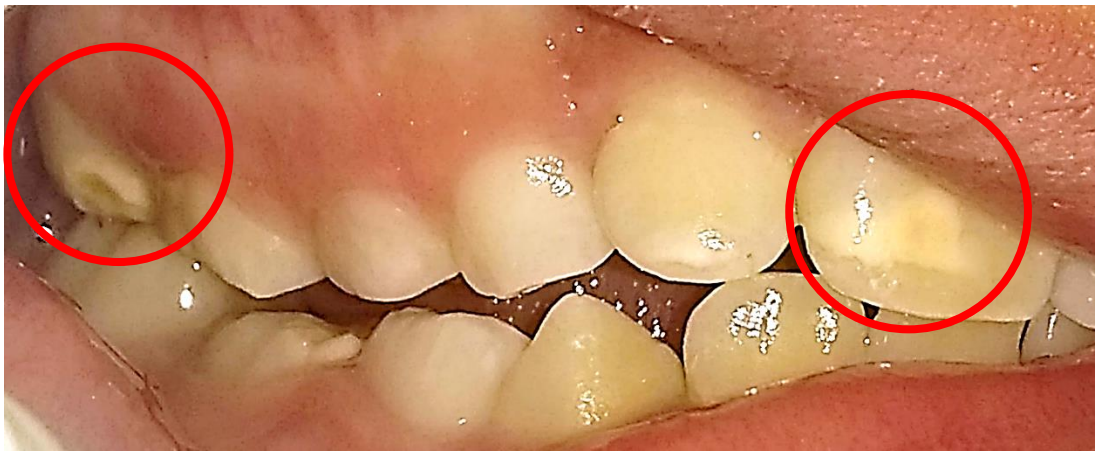

FIGURA 1. Dentição mista com presença de HMI em molar e incisivo permanentes. *Fonte: Autoria própria*

A recente prevalência de HMI encontrada no Brasil é de 12,1% em Curitiba (REYES et al., 2019), 14,5% na clínica de Odontopediatria da Universidade Federal do Rio de Janeiro (SILVA et al., 2020), 28,7% em Petrópolis-RJ (REIS et al., 2021). Mundialmente varia de 0,48% a 37,9 % de acordo com estudos prévios (SUBRAMANIAM et al. 2016, ILCZUK-RYPUŁA et al. 2022, NISII et al., 2022, VANHÉE et al. 2022, VILLANUEVA-GUTIÉRREZ et al. 2019, AFSHARI et al. 2022, KÜHNISCH et al. 2014).

Uma recente revisão sistemática com metanálise ressaltou, ao incluir 116 estudos, que mundialmente, a prevalência de HMI foi em média de 13,5% (IC 95% 12,0–15,1, I 2 = 98,0%). A América foi o continente com maior prevalência (15,3, IC 95% 12,8–18,3, p < 0,001, I 2 = 96,3%) e a Ásia com a menor (10,7, IC 95% 8,5–13,5, p < 0,001, I 2 = 98,7%). Contudo, não foram encontradas diferenças continentais (LOPES et al., 2021).

A amelogênese, processo de formação do esmalte, apresenta alta sensibilidade e é controlada geneticamente, sendo desafiador prevenir os fatores que podem afetar estas células formadoras do esmalte, resultando na HMI (FIGURA 2). A amelogênese dos primeiros molares e incisivos permanentes inicia-se no período final da gestação, e finaliza-se por volta dos três a cinco anos de idade (GUEDES-PINTO, 2016). Sugere-se que episódios de privação de oxigênio, os quais ocorrem em patologias respiratórias como asma, também podem provocar efeitos deletérios na amelogênese (KÜNISCH et al., 2014, TOURINO et al., 2016, DE LIMA et al., 2015).

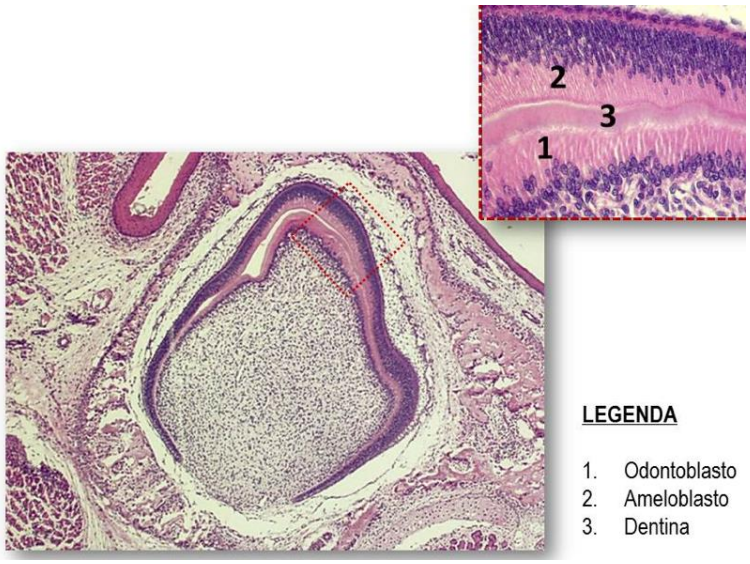

FIGURA 2. Histologia da formação de um elemento dentário. *Fonte: https://histobuco.paginas.ufsc.br/amelogenesedentinogenese*

Ao iniciarem a erupção na cavidade bucal, por volta dos 6 anos, os dentes apresentam-se em infra oclusão, tendo mais chances de serem acometidos pela cárie (FRAGELLI et al., 2015), pois mesmo em condições normais os dentes em erupção ainda não passaram pela maturação pós-eruptiva, a qual completa-se cerca de dois anos depois de estarem em boca (FIGURA 3) (GUEDES-PINTO, 2016).

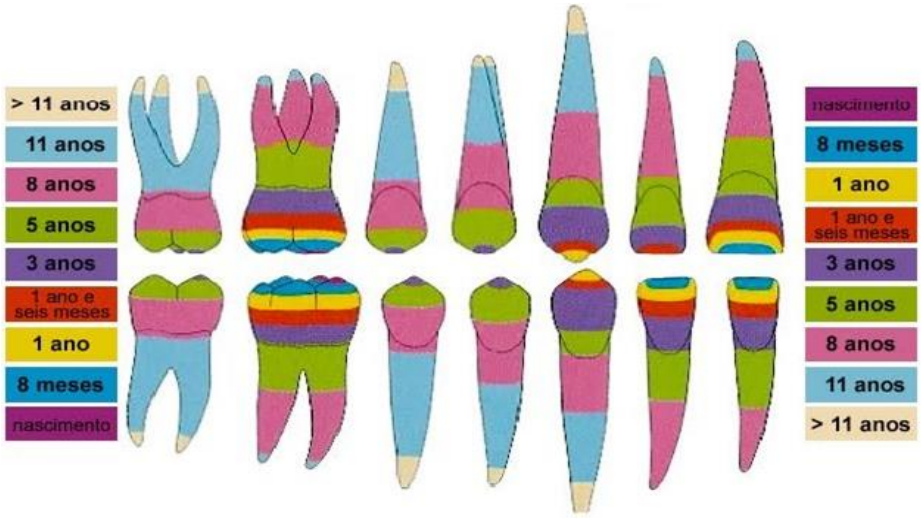

FIGURA 3. Idade cronológica da formação/mineralização dos dentes permanentes. *Fonte: PINTO LO, FRAGELLI C, IMPARATO J. Hipomineralização de molares e incisivos. 1ª Edição. SP: Napoleão 2020.*

Adicionalmente, crianças com manifestações de doenças respiratórias nos primeiros quatro anos de vida apresentaram 2,48 vezes mais chances de desenvolver HMI envolvendo molares e incisivos simultaneamente (KÜNISCH et al., 2014). Patologias como bronquite e asma podem provocar situações de privação de oxigênio, os quais podem causar efeitos deletérios na fase de amelogênese (KÜNISCH et al., 2014, TOURINO et al., 2016, DE LIMA et al., 2015). Além disso, o artigo de revisão sistemática apresentado como capítulo 1 nesta tese concluiu que a asma está intimamente ligada a defeitos de esmalte em dentes permanentes jovens (RIZZARDI et al., 2022). A alta frequência de exposição das crianças a penicilinas, cefalosporinas e macrolídeos, acontecem devido ao grande índice de infecções na primeira infância, as quais podem gerar impactos negativos na formação de esmalte, tendo como causa a doença infecciosa ou diretamente a antibioticoterapia (TARIQ et al., 2014, WUOLLET et al., 2016).

A asma é uma doença inflamatória crônica caracterizada por hiperresponsividade das vias aéreas inferiores e limitação variável do fluxo aéreo (REZENDE et al., 2019) que está intimamente relacionada à privação de oxigênio (KÜHNISCH et al. 2014, DE LIMA et al., 2015). Em crianças com asma, hipóxia e hipoventilação são comuns. Baixos níveis de oxigênio podem ter efeitos

deletérios na amelogênese. Nesse processo, as células do epitélio interno do órgão do esmalte se diferenciam em ameloblastos, responsáveis pela síntese do esmalte na coroa do futuro dente. Na fase de secreção, a matriz do esmalte é formada e, na fase de maturação, a matriz é progressivamente mineralizada. Níveis anormais de oxigênio e acidose respiratória afetam o pH da matriz do esmalte e, como resultado, a ação de enzimas proteolíticas é inibida. Dessa forma, as proteínas retidas não fornecem espaço para adequada deposição mineral (FARAH et al. 2010), prejudicando o desenvolvimento do cristal de hidroxiapatita, que é de suma importância para um esmalte saudável. No processo de mineralização do esmalte, para cada nova célula unitária de hidroxiapatita, oito íons de hidrogênio são liberados (SIMMER; FINCHAM, 1995). Como as condições de pH neutro ou quase neutro são cruciais para a deposição mineral adequada, a remoção dos íons de hidrogênio liberados pela hidroxiapatita é um pré-requisito básico (SUI; BOYD; WRIGHT 2003). Em um ambiente ácido, a amelogenina, bem como outras proteínas da matriz do esmalte, são alteradas. Além disso, quando o pH da matriz do esmalte atinge níveis críticos, ocorre perda mineral (SUI; BOYD; WRIGHT 2003), favorecendo os defeitos do esmalte e a hipomineralização.

O dente acometido por HMI pode apresentar dentina exposta desde a sua erupção na cavidade bucal, tendo assim a coloração alterada de branca até o amarelo amarronzado podendo apresentar-se sem proteção do esmalte (LAGO et al., 2017), pois o esmalte defeituoso pode ficar extremamente susceptível a fratura (DE OLIVEIRA et al., 2015), em função das forças mastigatórias, o que propicia:

- Sensibilidade: os prolongamentos dos odontoblastos estão alojados nos túbulos dentinários, são células vivas localizadas na polpa que apresentam-se fora da organização habitual;

- Acúmulo de biofilme: em função das cavidades e microcavidades formadas pelas irregularidades e retentividades da superfície.

- Processo carioso severo: dentes facilmente atingidos, pois a dentina apresenta-se exposta e seu pH de desmineralização (6,5) é inferior ao do esmalte (5,5 para a hidroxiapatita e 4,5 para a flúorapatita) (FEJERSKOV; KIDD, 2011).

Além disso, existe uma pré-disposição a lesões de cárie secundárias nos elementos dentários já tratados, pois o esmalte alterado pela HMI já propicia uma superfície retentiva com exposição dentinária.

A cárie infantil é uma doença multifatorial, que apresenta prevalência significativa em escolares brasileiros, variando de 15,25 a 88,7% em dados atuais (DIAS; MARQUES, 2018, GOMES et al., 2022, MOIMAZ et al., 2022, GRANJA et al., 2022, VOLLÚ et al., 2022, LIRA et al., 2022, TSUZUKI et al., 2018). As lesões de cárie iniciam-se por meio da desmineralização dos tecidos dentários (GROSSI; CABRAL; LEAL, 2017) devido à ação de ácidos orgânicos originados da fermentação bacteriana de substratos alimentícios, principalmente os carboidratos (SELWITZ, 2007).Um fator de grande preocupação nas crianças para o desenvolvimento da doença cárie é o alto consumo de açúcares livres presentes, por exemplo, nos sucos de fruta industrializados e lanches os quais são adicionados pelo fabricante, cozinheiro ou consumidor (KEYES, 1960, SELWITZ, 2007, DO NASCIMENTO LEITE; DA SILVA DAMACENO; LOPES, 2021). A presença e contagem de *Lactobacillus* spp. é um bom indicador do consumo recente de açúcar, ou seja, mudanças no consumo de açúcar podem ser evidenciadas nessa contagem. Alterações microbiológicas são promovidas pela sacarose, a qual atua como substrato específico para a produção de glucanos insolúveis que aumentam a viscosidade desse biofilme, facilitando a aderência de microrganismos patogênicos da cárie, como *S. mutans e Lactobacillus* spp. (NOBRE-DOS-SANTOS et al., 2002, PAES LEME et al., 2006, PARISOTTO et al., 2010b, WHO 2015, AIRES et. al., 2006). Inúmeros estudos apresentam maior contagem de tais patógenos em crianças com cárie (MATTOS-GRANER et al., 1998, NOBRE-DOS-SANTOS et al., 2002, BARSAMIAN-WUNSCH et al., 2004, PARISOTTO et al., 2010), sendo diretamente relacionados ao desenvolvimento e progressão das lesões cariosas tanto em dentes hígidos como nos acometidos pela HMI.

O desenvolvimento de lesões cariosas de avanço rápido pode mascarar as superfícies hipoplásicas e hipomineralizadas, considerando-se o maior risco de cavitações de uma estrutura com HMI (DA COSTA–SILVA et al., 2011). Devido à presença dessas lesões, as alterações de esmaltes podem não ter a devida importância no diagnóstico, havendo uma sub-notificação dos casos (MITTAL et al., 2016). Quanto mais molares permanentes apresentam HMI, aumentam as chances de acometer também os incisivos, o que pode relacionar-se a problemas estéticos (MURATBEGOVIC et al., 2007).

A avaliação do desenvolvimento das comunidades bacterianas com potencial cariogênico nos indivíduos com HMI é interessante para a identificação da presença de bactérias capazes de promover maior dissolução do esmalte dentário nesta condição. A literatura científica evidencia

poucas pesquisas, na atualidade, envolvendo HMI e microbiota bucal (FAGRELL et al., 2008, KOLEVENTI et al., 2018) e quando essas condições foram associadas a metodologias de biologia molecular verificou-se apenas dois artigos na base de dados PUBMED (JEREMIAS et al., 2013, HERNÁNDEZ et al., 2020).

Para a identificação da composição da microbiota bucal humana, técnicas moleculares independentes de cultivo têm evidenciado uma microbiota muito diversificada. Uma ferramenta muito eficaz na determinação da inter-relação evolucionária dos microrganismos é a análise de sequenciamento do gene RNA ribossomal (RNAr) (BLAUT et al., 2002), o qual apresenta regiões de variabilidade específicas para grupos e espécies, assim como regiões que são encontradas em todas as bactérias (FURRIE, 2006). A caracterização da microbiota humana tem sido amplamente utilizada com a construção de bibliotecas do gene 16S nos últimos anos (HAYASHI et al., 2005; STRAUSBERG et al., 2008; LI et al.; 2012; LIN et al., 2013; DURBÁN, et al., 2011; REHMAN et al., 2010; WALKER et al., 2011; SHEN et al., 2010, HERNÁNDEZ et al., 2020), visto que para se conhecer a filogenia dos procariotos o gene 16S tem sido o alvo mais empregado (BLAUT et al., 2002). Apesar de ser uma metodologia cuidadosa e de custo significativo o entendimento do microbioma, pode trazer informações adicionais que certamente contribuirão para a prevenção tanto da cárie como da HMI.

A relação entre cárie dentária e HMI já foi evidenciada em crianças brasileiras (JEREMIAS et al., 2013) e o estudo recente de Oreano et al. (2022) mostrou que a presença de HMI foi associada 6,15 vezes com uma maior prevalência de cárie nos primeiros molares permanentes. Os autores Grossi, Cabral, e Leal (2017) também mostraram que dentes com HMI apresentam cárie dentária com maior frequência e que a progressão da lesão cariosa na presença dessa condição dá-se de forma rampante e em maior velocidade, quando comparados a dentes não comprometidos por HMI (GROSSI; CABRAL; LEAL, 2017).

O controle da cárie em dentes acometidos por HMI é um grande desafio na clínica odontopediátrica (OREANO et al., 2022). O esmalte do dente afetado por HMI apresenta diferenças quando comparado ao esmalte normal, sendo assim, um melhor prognóstico do elemento dentário acometido por HMI depende do seu diagnóstico precoce (MELIN et al., 2015).

O tratamento da HMI pode variar com a erupção do dente, a colaboração da criança, a severidade da hipomineralização e o comprometimento familiar com o tratamento. Ao realizar a

identificação da HMI, medidas preventivas e de mínima intervenção devem ser empregadas, isso porque, com o diagnóstico tardio (WEERHEIJM, 2004) o trabalho clínico torna-se mais complicado, visto que esses dentes apresentam dificuldades de serem anestesiados devido a inflamação subclínica das células pulpares pela porosidade causada no esmalte, tornando-os permeáveis às toxinas microbianas (OYEDELE et al., 2015). O controle da dor é indispensável no atendimento odontopediátrico, fazendo destes casos um desafio para os dentistas (KALKANI et al., 2016).

É importante enfatizar que tratamentos preventivos geram custos menores ao sistema de saúde, sendo de grande relevância para países como o Brasil, em processo de desenvolvimento. Cirurgiões dentistas brasileiros precisam cada vez de mais informação, visto que ainda são adeptos ao tratamento curativo, o qual não combate os fatores etiológicos das doenças. Estas mudanças potencializariam as boas condições de saúde, alteram hábitos que predispõem patologias (GAMBETTA-TESSINI et al., 2016) fundamentais para um bom prognóstico.

Fica explícito que a cárie infantil ainda apresenta alta prevalência na população escolar e que a HMI vem ganhando maior relevância nos tempos atuais. O desenvolvimento de estudos direcionados aos fatores etiológicos destas condições são importantes visto que o assunto é de expressão mundial e que a literatura científica abordando conjuntamente cárie HIM é escassa considerando-se os aspectos microbiológicos.

2. Objetivos

2.1. Objetivo Geral

O objetivo do presente estudo foi revisar a literatura no que diz respeito a defeitos de esmalte e asma e especialmente no estudo transverso avaliar a relação entre os diversos estágios de HMI e a presença de bactérias cariogênicas em escolares bragantinos de 6 a 8 anos.

2.2. Objetivos Específicos

- Revisar sistematicamente artigos sobre a associação entre asma e defeitos de esmalte em dentes decíduos e permanentes jovens.

- Comparar as bactérias presentes na cavidade bucal no grupo de crianças com primeiros molares permanentes saudáveis e os grupos experimentais com HMI.

3. CAPÍTULO I – Artigo publicado no periódico "*Pediatric Pulmonology"*
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As doenças respiratórias infantis, como a asma, são importantes problemas de saúde pública em todo o mundo e podem estar associadas a defeitos no esmalte dentário. Dentes com defeitos significativos em sua estrutura, como os acometidos pela Hipomineralização de Molar-Incisivo (HMI), são mais susceptíveis ao acúmulo de biofilme e às lesões cariosas. Assim, pesquisas que abordem asma e HMI conjuntamente devem ser estimuladas. Este capítulo da tese tem o objetivo de revisar sistematicamente artigos sobre a associação entre asma, seus medicamentos e defeitos de esmalte nos dentes, para responder à seguinte pergunta: "A asma em crianças pode estar significativamente associada a defeitos de esmalte na dentição decídua e nos dentes permanentes jovens?" No levantamento dos artigos da revisão (2000-2021) foram cuidadosamente pontuados de acordo com um critério predeterminado, os Itens Preferenciais de Relatórios para Revisões Sistemáticas e Meta-Análises foram considerados e foram incluídas quatro bases de dados (PUBMED-MEDLINE, EMBASE, LILACS, COCHRANE). Os dados da revisão foram avaliados qualitativamente. Vinte e dois artigos foram criticamente avaliados, e a relação da asma e defeitos de esmalte foi encontrada em 1 de alto nível de evidência, 9 com nível moderado e 4 com nível baixo. A maioria dos estudos disponíveis para responder à questão abordada era do tipo transversal, coorte, randomizado controlado e caso-controle; portanto, mais investigações prospectivas de coorte bem projetadas são necessárias para confirmar nossos achados. Por meio da revisão conclui-se que asma está intimamente associada a defeitos de esmalte em dentes permanentes jovens.

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REVIEW

WILEY

Association between asthma and enamel defects in primary and young permanent teeth – A systematic review

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Abstract

Childhood respiratory diseases, such as asthma, are important public health problems worldwide and could be associated with tooth enamel defects. This study aimed to verify the relationship between asthma and enamel defects in teeth, to answer the following question: "Could asthma in children be significantly associated with enamel defects in deciduous dentition and young permanent teeth?." PUBMED-MEDLINE, EMBASE, LILACS, and COCHRANE databases were systematically searched and assessed articles (2000–2021) were cautiously scored according to a predetermined criterion. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses were considered. Twenty-two articles were critically appraised and used as a basis for conclusions. The relationship between asthma and enamel defects was confirmed in the majority of appraised papers, one with a high level of evidence, nine with a moderate level, and four with a low level. Out of the eight manuscripts investigating the influence of asthma medication on enamel defects, only three (one with high, one moderate, and another with a low level of evidence) suggested an association. It can be concluded that asthma is closely connected with enamel defects in young permanent teeth. However, as most of the papers appraised were of cross-sectional or case-control design, further well-designed clinical investigations with a prospective design are welcome to reinforce our findings.

KEYWORDS
asthma, enamel defects, hypomineralization, molar incisor hypomineralization

1 | INTRODUCTION

Enamel defects may be evidenced in children at the beginning of primary or mixed dentition, and factors related to general health could contribute to this condition.[1] The process of dental enamel formation is denominated amelogenesis. Enamel is the most resistant and mineralized tissue in the human body, composed of 97% minerals and 3% water and organic material, such as proteins (e.g., enamelin and amelogenin). The deposition of tooth enamel is structured in two stages: (i) the secretory stage, in which proteins and an organic matrix form a partially mineralized enamel; and (ii) the maturation stage, in which the mineralization of the tissue is completed.[2,3] The amelogenesis process is highly sensitive and genetically controlled, influenced by general health conditions, and affects the enamel-forming cells.

While primary teeth are formed intra-uterus, the amelogenesis of the permanent first molars and incisors begins in the last pregnancy trimester and ends at approximately 3–5 years of life.[4] A study involving Thai children indicated that the method of delivery (cesarean

section or complicated vaginal delivery), as well as a severe and chronic illness at the age of 3 years, was strongly associated with molar incisor hypomineralization (MIH),[5] a specific condition affecting permanent molars and/or incisors. The problems likely occur at the time of enamel maturation in the amelogenesis process, affecting the tooth structure. Enamel defects in the deciduous dentition indicate intercurrences in the prenatal or early postnatal period that could be responsible for altered matrix formation due to damaged ameloblasts during enamel maturation.[6]

Remarkably, problems such as acidosis, hypoxia, and hypocalcemia could stimulate the biological mechanisms involved in the development of hypoplasia and hypomineralization.[7,8] Teeth affected by these defects might show changes from white to brownish-yellow and failed enamel layers, leading to exposed dentin, once erupted into the oral cavity.[9] Exposed dentin is intimately related to sensitivity, and the pH of dentin demineralization (pH = 6.5) is higher than that of enamel (pH = 5.5 for hydroxyapatite and 4.5 for fluorapatite), favoring caries development.[10] It is also important to highlight that the defective enamel in these cases shows irregularities and retentivity and is susceptible to fracture[11] due to masticatory forces. In this scenario, biofilm accumulation and early carious lesions are enhanced. Moreover, the development of lesions is rapid and may mask hypoplastic and hypomineralized surfaces.[12]

Studies have shown that children with respiratory diseases, such as asthma, pneumonia, or upper respiratory tract infections,[8,13–15] are prone to developing tooth defects. In the first 4 years of life, children affected by respiratory illness have 2.48 times more chance of developing MIH.[13] In addition, individuals with any chronic and/or serious disease (e.g., pneumonia, asthma, high fever, tonsillitis, chickenpox, measles, and seizure) are two times as likely to have enamel alterations (odds ratio [OR] = 2.2).[5] The asthma incidence has progressively increased in recent decades, being the most common chronic disease in children and adolescents. Specific etiological factors and mechanisms of asthma pathogenesis have not been elucidated; however, asthma is understood as an umbrella term for many phenotypes, originating from distinct pathophysiological pathways.[16]

Studies concerning the etiology of enamel defects are scarce in the literature, and high-quality clinical studies should be stimulated. Furthermore, literature reviews with systematic methods assessing and summarising existing data play a pivotal role in identifying gaps for further investigations. Thus, this study aimed to undertake a systematic review to verify the relationship between asthma and enamel defects in deciduous and young permanent teeth.

2 | MATERIALS AND METHODS

2.1 | Question addressed by present review

"Could asthma be significantly associated with enamel defects in deciduous dentition and young permanent teeth?"

2.2 | Literature search

A comprehensive literature search was performed in the PUBMED-MEDLINE, EMBASE, LILACS, and COCHRANE databases in June 2021, assessing articles published in the previous 21 years. A manual search was also performed. The Preferred Reporting Items for Systematic Reviews (PRISMA) protocol was followed. Considering the aim of the present systematic review, the following search descriptors (i.e., MESH-terms) and text words were used: "chronic disease" and "asthma," one at a time, along with: "hypomineralization," "enamel defect," and "molar incisor hypomineralization." The full detailed search strategy is available in Supplement S1. The search was limited to longitudinal or cross-sectional investigations, case-control studies, cohort or randomized controlled trials, comprising relevant records addressing the relationship between asthma and enamel defects.

The minimal requirements for inclusion were studies evaluating tooth defects, as an outcome, and the presence of asthma or asthma medication in childhood, exploring the interrelationships between them. The asthma diagnosis includes self-reports or medical records. The tooth defects were diagnosed by dentists, including MIH, deciduous molar hypomineralization, and enamel imperfections in general, in both primary and permanent teeth. Single or multiple defects, regardless of size and location (buccal and lingual tooth surfaces), were commonly characterized by: demarcated opacities (white or yellow—single or multiple); diffuse opacities (parallel lines or patchy distribution); and hypoplasia (pits, grooves, or larger areas of missing enamel—single or multiple).

Initial databases search yielded 458 papers and after duplicates or triplicates, removal 301 records were screened. After exclusions due to publications before the year 2000, clinical guidelines, papers in languages other than English, animal subjects, literature reviews and manuscripts unrelated to the question addressed (i.e., papers not focusing on the interplay between asthma and enamel defects), 230 reports were assessed for eligibility, and at last 22 were included in the qualitative synthesis (Figure 1). These 22 full-text articles were carefully appraised and used as a basis for conclusions.

2.3 | Evaluation of scientific articles and levels of evidence

Articles that met all the inclusion criteria were subjected to critical evaluation by two independent researchers (Karina F. Rizzardi and Thaís M. Parisotto). The evaluation criterion was standardized, and any disagreement between the reviewers was resolved by a discussion between them until a consensus could be reached. Each selected article was scored from grades A to C based on Indiani et al.[17] (Table 1). Manuscripts addressing all issues described in grade A, such as the full description of eligibility criteria and sample selection, stratification of children, evaluation of enamel defects with a validated methodology criterion, calibration of the examiner (including agreement levels), confounders considered, medical register-based asthma diagnosis or medical/drugstore register-based asthma medication, and adequate

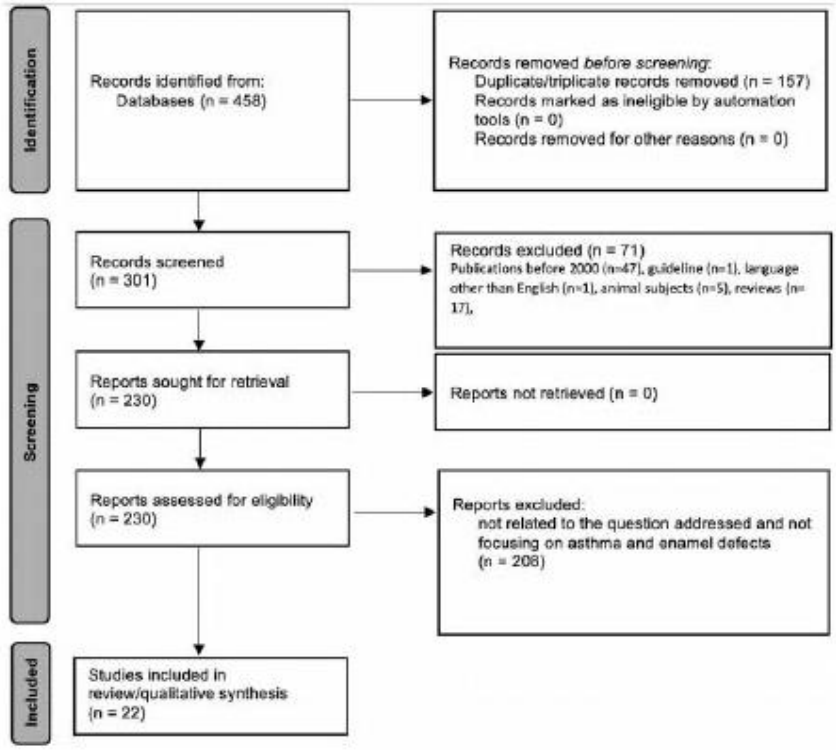

FIGURE 1 Flowchart of the selected studies

statistical analysis applied to present the results in tables or graphs, were rated as having a high value of evidence.

Conversely, when the eligibility criteria and sample selection were not fully described, stratification and calibration were not performed in detail, confounders were not included in the discussion section, and asthma diagnosis/medication was based on recall articles were scored as grade B, having moderate value as evidence. Finally, when the eligibility criteria and sample selection were poorly described, when confounders, the method for enamel defect diagnosis, and statistical analysis were not reported, and asthma was considered jointly with bronchitis, a score of grade C was given, referring to a poor level of evidence.

3 | RESULTS

3.1 | Studies selection and scoring

The electronic literature search resulted in 458 articles, of which 157 duplicates/triplicates were removed. Manual handling assessment was used to include only original manuscripts published after the year 2000, written in the English language, without animal subjects. This process resulted in 230 articles. Of these, 208 were excluded or were not related to the question addressed, leading to 22 studies being critically appraised and used as the basis for a conclusion (Figure 1).

The studies by Viweswar et al.,[18] (Table 2), Elfrink et al.,[19] Mastora et al.,[20] and Wogelius et al.[21] (Table 3), were classified as "grade A" category, as they employed medical records for asthma diagnosis or medical/drugstore report for asthma medication registration. Furthermore, they used valid criteria for enamel defects detection, described in full the inclusion and exclusion criteria and the sampling strategy. The studied groups were categorized according to age or sex, confounders were considered, examiners were calibrated, and statistical tests were performed (Table 1).

Despite presenting the characteristics described above, the study by Ferrazzano et al.[24] was rated as "grade B" because the results of a significant analysis (logistic regression analysis) considering enamel defects and asthma was only briefly mentioned in the text, without visual representation (table/graph) and an indication of effect size, confidence interval (CI), and *p* values.

The articles by Jalevik et al.,[38] Ford et al.,[22] Guergolette et al.,[23] Durmus et al.,[25] Allazzam et al.,[26] Pitiphat et al.,[5] Koruyucu et al.,[29] Lima et al.,[32] Flexender et al.,[37] Neto et al.,[34] and Wogelius et al.[35] (Tables 2 and 3), were rated as "grade B" since they considered self-report for asthma diagnosis or asthma medication registration. Answers to interview or questionnaire questions were designated as self-reports. In the papers mentioned above the inclusion and/or exclusion criteria, sample selection, calibration of the examinator, or presence of bias were not fully described (Table 1).

TABLE 1 Criteria for grading assessed scientific articles

Grade	
A	Inclusion and exclusion criteria described
	Methods of sample selection completely described
	Stratification by sex and age of subjects
	Calibration of the examinator describing agreement levels, i.e., Kappa
	Enamel defects/asthma evaluated as outcomes
	Valid method for enamel defects diagnosis described
	Medical register-based asthma diagnosis
	Medical/drugstore register-based asthma medications
	Bias or confounders were taken into account
	Statistical analysis applied and results shown in tables or graphs
B	Inclusion and exclusion criteria not completely described
	Methods of sample selection not described in detail
	Partial stratification by sex and age of subjects
	Calibration of the examinator without describing agreement levels
	Enamel defects and asthma were evaluated as outcomes
	Method for enamel defects diagnosis not completely described
	Recall-based asthma diagnosis
	Recall-based asthma medications registration
	Bias or confounders not completely taken into account
	Statistical analysis applied
C	Inclusion and exclusion criteria poorly described
	Methods of sample selection poorly described
	No stratification by sex and age of subjects
	No calibration of the examinator
	Enamel defects evaluated
	Methods for enamel defects diagnosis poorly described
	Recall-based asthma diagnosis together or not with bronchitis
	Recall-based asthma medications registration
	Bias or confounders not taken into account
	Statistical analysis not described

The following four manuscripts were classified as a poor value of evidence (grade C – Table 1), Hernandez et al.,[28] Hernandez et al.,[33] Kilinc et al.,[31] and Loli et al.[36] (Tables 2 and 3) because they considered as outcome asthma jointly with bronchitis, did not specify the diagnosis criteria for the enamel defects, did not mention examiner calibration, eligibility criteria were not well described or bias/confounders not taken in account (Table 1).

The studies of Tourino et al.[27] and Rezende et al.[30] investigated the relationship between asthma and enamel defects, as well as between asthma medication usage and enamel defects. In the research conducted by Rezende et al.,[30] asthma diagnosis was confirmed by a doctor according to medical records, but the use of asthma medication was estimated only by questionnaire (self-report). For these reasons, this study was rated as A (Table 2) regarding asthma disease and enamel defects and B (Table 3) concerning asthma medication usage and enamel defects. In the population-based study conducted by Tourino et al.,[27] children were evaluated for asthma jointly with bronchitis, and the medical history, as well as medicine usage information, was obtained by questionnaire (self-report). Thus, this study was rated as C (Table 2) and B (Table 3) respectively, considering the impact of asthma disease on enamel defects and the impact of asthma medication on enamel defects.

Asthma/asthma drugs and their relationship with general defective enamel were confirmed in five investigations, including three with a moderate and two with a high level of evidence (Table 2). The association between asthma and MIH specifically was found in ten studies, six of which were rated as grade B and four as grade C (Table 2). Also, the connection between asthma drugs and general defective enamel/MIH was shown in three papers, one rated as high, one rated as medium, and one rated as low level of evidence (Table 3).

However, the lack of association between asthma or asthma drugs and general defective enamel or MIH/DMH was pointed out by Ferrazzano et al.[24] (grade B), Rezende et al.[30] (grade A/B), Neto et al.[34] (grade B), Wogelius et al.[35] (grade B), Elfrink et al.[19] (grade A), and Wogelius et al.[21] (grade A; Tables 2 and 3). The study of Flexeder et al.[37] (grade B) did not show an association only between asthma drugs and MIH (Table 3).

All 22 articles fully appraised were used as a basis for conclusions.

3.2 | Asthma disease and enamel defects

A positive connection between asthma and enamel defects in children was found in 14 papers, out of 17 exploring this relationship, most of them rated as the moderate value of evidence (Table 2).

The study of Viweswar et al.[18] (grade A) involved a cross-sectional design, with 208 Indian children (7–14 years) equally assigned into asthma and control groups. The asthmatic patients were recruited from pediatric chest clinics after confirmed asthma diagnosis by a doctor/pediatrician. The risk of enamel defects in the permanent dentition was 12 times higher in children with asthma than those without (OR: 12.52, $p = 0.001$). Likewise, a cross-sectional study involving 136 children and adolescents (5–15 years of age) allocated into two groups (asthma $n = 68$; and control $n = 68$), was conducted in the urban area of the south of Brazil.[23] According to logistic regression analysis, the risk of enamel defects in the permanent dentition was 11 times higher in pediatric subjects with asthma than those without (OR: 11.88, CI: 4.38–32.19, $p = 0.0001$), and the occurrence of defects correlated positively with the severity of the

TABLE 2 Results of scientific articles appraised regarding asthma disease

	Study design	First author	Year	Geographic location	Number of subjects	Age of subjects (years)	Significant direct association between asthma and enamel defects	Respiratory diseases assessed	Criteria for asthma diagnosis	Type of enamel defect assessed	Criteria for enamel defects diagnosis	Evidence value
01	Cross-sectional	Jalevik[19]	2001	Sweden (Urban area)	516	8	YES OR = 24 (CI: 5.2 ± 110) $p < 0.05$	Asthma	Self-report	MIH	DDE	B
02	Case-control	Ford[22]	2009	Australia (Urban area)	313	10–13	YES RR = 2.6 (CI: 1.2–5.3) $p = 0.007$ RR = 2.0 (CI: 1.0–3.9) $p = 0.04$	Asthma	Self-report	EDG in permanent teeth	DDE	B
03	Cross-sectional	Guergolette[23]	2009	Brazil (Urban area)	136	5–15	YES OR = 11.8 (CI: 4.38–32.19) $p = 0.0001$	Asthma	Self-report	EDG in permanent teeth	DDE	B
04	Case-control	Ferrazzano[24]	2012	Italy (Urban area)	280	7–10	NO $p > 0.05$	Asthma	Doctor report	EDG in primary permanent teeth	DDE	B
05	Cross-sectional	Viswesswar[18]	2012	India (urban area)	208	7–14	YES OR = 12.5 $p = 0.001$	Asthma	Doctor report	EDG in permanent teeth	DDE	A
06	Randomised controlled trial	Durmus[25]	2013	Turkey (Urban area)	228	7–14	YES $p = 0.05$	Asthma	Self-report	MIH	EAPD	B
07	Cross-sectional	Allazzam[26]	2014	Saudi Arabia (Urban area)	267	8–12	YES $\chi 2 = 31.477$ $p = 0.001$	Asthma	Self-report	MIH	EAPD	B
08	Cross-sectional	Pitiphat[5]	2014	Thailand (Urban area)	282	7 and 8	YES PR = 2.0 (CI: 1.2–3.3) $p = 0.02$	Asthma	Self-report	MIH	EAPD	B
09	Cross-sectional	Tourino[27,a]	2016		1181	8 and 9	YES PR = 1.9 (CI: 1.45–2.56)	Asthma and bronchitis	Self-report	MIH	EAPD	C

(Continues)

TABLE 2 (Continued)

	Study design	First author	Year	Geographic location	Number of subjects	Age of subjects (years)	Significant direct association between asthma and enamel defects	Respiratory diseases assessed	Criteria for asthma diagnosis	Type of enamel defect assessed	Criteria for enamel defects diagnosis	Evidence value
				Brazil (Urban and rural areas)			$p < 0.001$					
10	Cross-sectional	Hernandez[28]	2018	Spain (Urban area)	705	6–14	**YES** OR = 5.3 (CI: 2.7–10.1) $p < 0.001$	Asthma and bronchitis	-	MIH	EAPD	C
11	Cross-sectional	Koruyucu[29]	2018	Turkey (Urban area)	1511	8–11	**YES** $p < 0.001$	Asthma	Self-report	MIH	EAPD	B
12	Cross-sectional	Rezende[30,a]	2019	Brazil (Urban area)	228	6–12	**NO** $p = 0.531$	Asthma	Self-report and medical record	EDG in primary and permanent teeth	WHO/ modified DDE	A
13	Cross-sectional	Kılınç[31]	2019	Turkey (Urban area)	1237	9 and 10	**YES** $p < 0.001$	Asthma and bronchitis	Self-report	MIH	EAPD + Own criteria[b]	C
14	Cross-sectional	Lima[32]	2020	Brazil (Urban area)	811	5	**YES** PR = 1.69 (CI: 1.01–2.85) $p = 0.049$	Asthma	Self-report	EDG in primary teeth	EAPD	B
15	Cohort	Flexeder[32,a]	2020	Germany (Urban area)	730	10 and 15	**YES** OR = 2.56 (CI: 1.03–6.37) $p = 0.043$	Asthma	Self-report	MIH	EAPD	B
16	Cross-sectional	Hernandez[33]	2020	Spain (Urban area)	102	8–12	**YES** OR = 1.7 (CI: 1.05–2.76) $p \leq 0.05$	Asthmatic bronchitis/ asthma	Medical record	MIH	EAPD	C
17	Cross-sectional	Neto[34]	2020	Brazil (Urban área)	152	2–5	**NO** OR = 0.53 (CI: 0.12–2.29) $p = 0.395$	Asthma	Self-report	EDG in primary teeth	DDE	B

Abbreviations: χ2, Chi-square; 95% CI, 95% confidence interval; DDE, Developmental Defects of Enamel; EAPD, European Academy of Paediatric Dentistry; EDG, Enamel defects in general; MIH: Molar-Incisor Hypomineralization in permanent teeth; OR: odds ratio; PR, prevalence ratio; RR, relative risk; WHO, World Health Organization.

[a]Studies also described in Table 3, as they also assessed the relationship between asthma medication and enamel defects.

[b]The presence of only demarcated opacities in one or several first molar and incisor teeth was defined as MIH1, and the presence of posteruptive breakdown, atypical restorations, and extracted teeth was defined as MIH2.[31]

TABLE 3 Results of scientific articles appraised considering asthma medication

	Study design	First author	Year	Geographic location	Number of subjects	Age of subjects (years)	Significant direct association between asthma medication and enamel defects	Criteria for asthma medication evaluation	Medicine/drugs for asthma	Type of enamel defect assessed	Criteria for enamel defects diagnosis	Evidence value
01	Cross-sectional	Wogelius[35]	2010	Denmark (Urban area)	647	6–8	NO $p > 0.05$	Pharmacy records	β2-agonists, corticosteroids	EDG in permanent teeth	EAPD	B
02	Population-based prospective cohort study	Elfrink[19]	2013	Netherland (Urban area)	6.690	5 and 6	NO OR = 1.06 (CI: 0.61–1.54) $p > 0.05$	Pharmacy records	Sympathomimetics and corticosteroids during pregnancy	DMH	EAPD	A
03	Retrospective case-control	Loli[36]	2015	Italy (Urban area)	182	6–13	YES OR = 3.19 (CI: 0.67–1.70) $p < 0.001$ Spearman (r) 0.27 $p < 0.05$	Dentist records	Corticosteroids, β2-agonists, and mucolytics	MIH	–	C
04	Cross-sectional	Tourino[27,a]	2016	Brazil (Urban and rural areas)	1181	8 and 9	YES PR = 1.85 (CI: 1.44–2.38) $p < 0.001$	Self-report	Asthma medication in general	MIH	EAPD	B
05	Retrospective case control	Mastora[20]	2017	Greece (Urban area)	140	6–12	YES OR = 5.56 (CI: 2.10–14.70) $p < 0.001$	Medical records	Bronchodilators and corticosteroids: β2-agonists, leukotriene inhibitor, corticosteroids, glucocorticoids	EDG in permanent teeth	DDE	A
06	Cross-sectional	Rezende[30,a],	2019	Brazil (Urban area)	228	6–12	NO $p = 0.722$	Self-report	β2-agonists, corticosteroids, glucocorticoids, systemic corticosteroids	EDG in primary/permanent teeth	WHO/modified DDE	B
07	Population-based Cross-sectional study	Wogelius[35]	2020	Denmark (Urban area)	1837	9	NO $p > 0.05$	Pharmacy records	β2-agonists, corticosteroids	MIH	EAPD	A

(Continues)

TABLE 3 (Continued)

	Study design	First author	Year	Geographic location	Number of subjects	Age of subjects (years)	Significant direct association between asthma medication and enamel defects	Criteria for asthma medication evaluation	Medicine/drugs for asthma	Type of enamel defect assessed	Criteria for enamel defects diagnosis	Evidence value
08	cohort	Flexeder[37,a]	2020	Germany (Urban area)	730	10 and 15	NO $p > 0.05$	Self-report	Asthma medication in general	MIH	EAPD	B

Abbreviations: χ2, Chi-square; 95% CI, 95% confidence interval; DDE, Developmental Defects of Enamel; EAPD, European Academy of Paediatric Dentistry; EDG, Enamel defects in general; MIH: Molar-Incisor Hypomineralization in permanent teeth; OR: odds ratio; PR, prevalence ratio; WHO, World Health Organization.

[a]Studies also described in Table 2, as they also assessed the relationship between asthma disease and enamel defects.

disease (r: 0.51, $p = 0.0001$) as well as with early symptom onset (r: 0.67, $p = 0.0001$).[23]

Five hundred sixteen 8-year-old-children, representing 90% of students in the second grade of two Swedish urban communities, took part in the research of Jälevik et al.[38] Even though asthma was definitively not very prevalent in the studied population ($n = 5$, ≅1%), a strong association with enamel defects was reported (OR = 24, CI: 5.2–110, $p < 0.05$). In a similar way, after examining 1329 schoolchildren, aged 10- to 13-year-old, in the urban area of Australia, 105 children without enamel defects were selected as controls and 208 with enamel defects as cases (104 with hypoplasia [52 boys and 52 girls] and 104 with opacities [52 boys and 52 girls]).[22] Respiratory infections and asthma, in specific, among 0- to 3-year-old children (RR: 2.6, CI: 1.2–5.3, $p = 0.007$) and 4- to 6-year-old students (RR: 2.0, CI: 1.0–3.9, $p = 0.04$) were suggested as risk factors for the development of enamel hypoplasia in the permanent molars, detected after the eruption of these teeth.[22]

Of interest in a randomized controlled trial, 228 children (104 females and 124 males aged 7–14 years) were examined in the Department of Pediatric Dentistry of a Turkish Dental School.[25] Fifty-four children (24 females, 30 males; mean age = 9.9 ± 1.7 years) were diagnosed with MIH and assigned into the case group, while 53 (25 females, 28 males; mean age of 10.08 ± 2.25 years) without enamel defects were assigned into the control group. Differences between groups were observed in the numbers of children who had asthma before the age of 3 years ($p = 0.050$). More recently, a significant association between asthma/bronchitis and different stages of MIH (including yellowish-brown eruptive breakdown, atypical restorations, and extracted teeth) was detected in 9- and 10-year-old children recruited from a Dental Clinic of a Turkish University.[31] Once again, children attending a Pediatric Dental Clinic ($n = 267$ – Faculty of Dentistry – Saudi Arabia) were assessed and the results reinforced that MIH was more prevalent in children with asthma disease ($p = 0.001/\chi^2$: 31.477).[26]

The cross-sectional prevalence study of Pitiphat et al.[5] involving 420 eligible 7- to 8-year-old Thai children revealed a significant relationship between asthma and MIH (PR: 2.0, CI: 1.2–3.3, $p = 0.02$). In line with these findings, Koruyucu et al.,[29] with a meaningful sample ($n = 1511$, 8–11 years, both sexes) also found asthma to be significantly associated with MIH (χ^2: 16.51, $p < 0.001$).

A representative population-based study of 5-year-old northeast Brazilian preschoolers from an urban area ($n = 811$) reported using Poisson regression that children with asthma in the first year of life had 69% (PR: 1.69, CI: 1.01–2.85, $p = 0.049$) higher prevalence of enamel defects in primary teeth.[32] Again, in Brazil, considering older children (8–9 years), a similar cross-sectional study was developed with a representative sample of 1181 schoolchildren in urban and rural areas of the southeast.[27] Poisson regression with robust variance was performed for the analysis of factors associated with MIH highlighting that subjects who experienced asthma/bronchitis in the first 4 years of life have a higher prevalence of MIH (adjusted PR: 1.93, CI: 1.45–2.56, $p < 0.001$).[27]

Seven hundred thirty children and adolescents from an ongoing German birth cohort study, involving a population of high socioeconomic level, revealed that at age of 15 years, of the 78 asthmatic

adolescents, 27% present enamel defects.[37] After adjusted regression analysis, patients with asthma were 2.56 times (OR: 2.56, CI: 1.03–6.37, $p = 0.043$) more likely to have teeth affected by MIH.[37] The definition of asthma in the study of Flexender et al.[37] was based on parental reports (questionnaire) of a doctor diagnosis for each year up to 15 years.

In the northeast urban area of Spain (Barcelona), a cross-sectional study comprising 705 children, aging from 6 to 14 years verified that MIH was significantly more prevalent among those who had bronchitis/asthma (OR = 5.3, CI: 2.7–10.1, $p < 0.001$).[28] The same group of authors with a sample of 102 children (55 boys and 47 girls), whose age was between 8 and 12 years, reinforced the association between MIH and asthmatic bronchitis/asthma (OR: 1.70, CI: 1.05–2.76, $p \leq 0.05$).[33]

Differently from the results described above, lack of association between asthma and enamel defects in children was supported by three manuscripts, most of them rated as the moderate value of evidence. The paper of Ferrazano et al.[24] comprised 280 children of box sexes, ages 7–10 years, being 124 asthmatic and 156 age-matched healthy children from an urban area of Italy. While asthmatic children were recruited from the Hospital Department of Pulmonology, the healthy ones were recruited from public schools. Although the enamel defects were more prevalent among children with asthma, a lack of statistical significance was found between the two groups (asthmatic subjects vs. healthy subjects). In addition, no evidence for an association between asthma and enamel defects ($p = 0.531$) was shown by the cross-sectional study of Rezende et al.,[30] by examining 228 children (6–12 years, 112 asthmatic and 116 non-asthmatic) after sample size calculation. These children were taken from a list of registered users in the largest public health service network in southern Brazil.[30] At last, Neto et al.,[34] in an epidemiological, cross-sectional study also including Brazilian children ($n = 152$, age = 2–5 years) demonstrated absence of relationship between the presence of asthma and enamel defects (Fisher exact test – $p = 0.764$, binary logistic regression – OR: 0.53, CI: 0.12–2.29, $p = 0.395$).

3.3 | Asthma medication and enamel defects

Among the eight papers evaluating the relationship between asthma medication and enamel defects, three suggested the existence of an association (one scored grade A, one scored grade B, and one scored grade C – Table 3).

A retrospective investigation involving a control ($n = 70$) and case group ($n = 70$ – used asthma drugs) of children aged 6- to 12-year-old, with similar sex and age distribution, revealed that children attending the Pediatric Pulmonary Clinic using asthma medicines, according to medical records, have 5.6 times more chances to show enamel defects compared to the ones in the control group (OR: 5.56, CI: 2.10–14.70, $p < 0.001$).[20] Besides, severe hypoplastic lesions with loss of the enamel tissue were a common outcome among the affected first permanent molars. Children were recruited in a Pediatric Pulmonary Unit and the Unit of Allergology, Asthma, and Inflammation of a Greek Children's Hospital (cases), as well as in the Postgraduate Pediatric Dental Clinic (controls). Asthma drugs were classified as: (1) inhaled (salbutamol) and oral (montelukast) bronchodilators, and (2) inhaled (fluticasone, budesonide) and oral (dexamethasone, prednisolone, methylprednisolone) corticosteroids.[20] In a similar line, the research of Loli et al.,[35] employing a retrospective case-control design with 182 patients equally assigned to the case (children with MIH – 51% boys) and control groups (children without MIH – 51% boys) suggested a positive weak/moderate Spearman correlation between aerosol therapy and MIH in the general child population (r: 0.278, $p < 0.001$) and in boys (r: 0.372, $p < 0.001$). Children aging 6–13 years were selected from an Italian Pediatric Dentistry Department of the Policlinico. Use of the aerosol therapy including corticosteroids, β2-agonists, and mucolytics was assessed by drug history.[36]

A cross-sectional study, with a representative sample of Brazilian schoolchildren ($n = 1181$, 8–9 years) from urban and rural areas, evidenced that the use of medication for asthma was associated with MIH (crude PR: 1.85, CI: 1.44–2.38, $p < 0.001$).[27] Intake of asthma medication, in general, was obtained using a translated and certified questionnaire addressing questions regarding prenatal, perinatal, and health characteristics of the child in the first 4 years of life. The self-administered questionnaire was adapted from Jälevik et al.[38]

Conversely, five of eight papers evaluating the relationship between asthma medication and enamel defects indicated a lack of association, all of them rated as "grade A" or "grade B" (Table 3).

An ongoing cohort study in an urban area from the Netherlands, considering children and their mothers, indicated that there was no association between the use of antiasthma medicines (OR: 1.06, CI: 0.67–1.70) during pregnancy and DMH, which is a defect involving only primary teeth. Children's primary teeth were evaluated at the mean age of 6.2 years ($n = 6690$), and data regarding maternal use of antiasthma (inhalation, sympathomimetics, and corticosteroids) medicines during pregnancy were obtained from drugstore records.[19] Another ongoing birth cohort study evaluating children/adolescents in Germany found 52 (7.1%) asthmatic participants at the age of 10 years, of whom only 12 (23%) did not take metered-dose inhaler medication; and 78 (10.7%) asthmatic participants at the age of 15 years, of whom 21 (27%) did not take metered-dose inhaler medication.[37] The results did not show that the intake of those drugs had an impact on MIH prevalence at the 10-year and the 15-year follow-up period ($p > .05$).[37]

In a population-based cross-sectional study, Danish children aged 9 years ($n = 1837$) were clinically examined, and MIH was recorded.[21] No association between inhaled asthma medication and the prevalence of MIH was found, even after adjusting for gender, antibiotics usage, maternal smoking, pre/perinatal complications, and hospital admission with asthma diagnosis ($p > 0.05$). Data regarding medication usage was obtained from pharmacy/drugstore records, and asthma drugs were classified as (1) inhaled β2-agonists, (2) oral β2-agonists, and (3) inhaled corticosteroids.[21] An earlier cross-sectional study of the same group[35] included younger children (6–8 years) from two Danish municipalities, in whom all four permanent first molars had erupted ($n = 647$). No significant association between inhaled asthma medication during the first 3 years of life and an increased risk of demarcated opacities were found ($p > 0.05$). The slight tendency of an association

between inhaled asthma medication with MIH lesions in a more severe stage was not significant too.

The cross-sectional study of Rezende selected 362 at random from a list of registered users in the largest public health service network in southern Brazil.[30] After examining 228 of those children aged 6–12 years, half asthmatic ($n = 112$) and half non-asthmatic ($n = 112$), the Chi-square test demonstrated no connection between types of asthma medication and enamel defects ($p = 0.722$). Data regarding asthma drugs usage was obtained by questionnaire, and the following was reported: salbutamol, salbutamol + beclomethasone or budesonide + systemic corticosteroids.[30]

4 | DISCUSSION

To the best of our knowledge, the present review is the first to systematically explore and critically appraise papers investigating the relationship between asthma/asthma medication and enamel defects in the child population. It is important to highlight that systematic reviews play an important role in assessing the quality of the available scientific evidence and guiding the development of well-designed studies to overcome gaps.

Regarding research evaluating the relationship between asthma and enamel defects, 82% (14 out of 17) supported the existence of an association (1 scored "grade A," 9 scored "grade B," and 4 scored "grade C" – Table 2) and most of them considered young permanent teeth.[5,18,22,23,25–29,31,33,37,38] Intriguingly, it is intrauterine that children's first permanent molars/incisors begin the amelogenesis process (from the cusps to the cervical direction of the teeth), which is completed at around 3–5 years of age.[4] Thus, during this period, systemic problems can affect the ameloblasts cells, which are highly sensitive. Different from other tissues, dental enamel, once formed, is not capable of remodelation. Therefore, enamel defects act as a biological record of systemic injuries.

Asthma is a chronic inflammatory disease characterized by lower airway hyperresponsiveness and variable airflow limitation,[30] which is closely related to oxygen deprivation.[39,40] In children with asthma, hypoxia, and hypoventilation are common. Low levels of oxygen may have deleterious effects on amelogenesis. In this process, cells of the enamel organ internal epithelium differentiate into ameloblasts, which are responsible for synthesizing enamel in the crown of the future tooth. In the secretion phase, the enamel matrix is formed, and in the maturation phase, the matrix is progressively mineralized. Abnormal oxygen levels and respiratory acidosis affect the enamel matrix pH, and as a result, the action of proteolytic enzymes is inhibited. In this way, the retained proteins do not provide space for adequate mineral deposition,[41] prejudicing hydroxyapatite crystal development, which is of prime importance for healthy enamel. In the enamel mineralization process, for every new unit cell of hydroxyapatite, eight hydrogen ions are released.[42] As neutral or near-neutral pH conditions are crucial for adequate mineral deposition, the removal of hydrogen ions released by hydroxyapatite is a basic prerequisite.[43] In an acidic environment, amelogenin as well as other enamel matrix proteins are changed. Moreover, when the pH of the enamel matrix reaches critical levels, mineral loss occurs,[43] favoring enamel defects and hypomineralisation. The proposed mechanisms may explain why asthma is associated with enamel defects, and, considering that asthma is the most common non-communicable disease in children, its association with these defects is worrisome.[44]

Regarding the two studies focusing on enamel defects in primary teeth[32,34] and asthma, divergent findings were found, because one of them pointed to the existence of a significant association,[32] and the other did not[34] (Table 2). Both involved preschool children from the northeast region of Brazil, but the research of Neto et al.[34] considered public schools only,[34] and the research of Lima et al.[32] considered private and public. Both diagnosed asthma as a self-report and used different index to evaluate enamel defects (DDE [Developmental Defects of Enamel] vs. EAPD [European Academy of Paediatric Dentistry] – Table 2). Distinctions in the sample characteristics as well as in the indexes may have played a role in explaining the different results.

Interestingly, in all of the papers that investigated asthma and MIH, a positive relationship was observed[5,25–29,31,33,37,38] (Table 2). MIH is a specific enamel malformation that affects young permanent teeth, without an enlightened etiology, and is influenced by general health problems during the amelogenesis process, genetic predispositions, or environmental factors.[9] Defects can be quite small, with marked opacities of white color, or severe, in which the supportive enamel is usually worn out and the tooth assumes a yellowish-brown coloration with high sensitivity.[45] Interest regarding MIH is increasing outstandingly in the last years, as reflected by the number of publications at PUBMED central.

Considering asthma drugs concerning enamel defects, it was already reported that glucocorticoids therapy may lead to diminished bone formation, by inducing osteoclast apoptosis and increasing osteoclast survival and activity.[46] Therefore, it might be supposed that the action of corticoids, particularly amounts accumulated in the mouth after aerosol therapy, may play a role in the formation of teeth lesions, by a similar impact on the ameloblasts cells. However, asthma drugs and their relationship with defective enamel were not shown in most of the investigations assessed in this regard (37%, three out of eight papers). It seems that asthma disease could exert a higher impact on ameloblasts than the medication themselves, due to hypoxia. Remarkably, the cohort study of Flexender et al.[37] revealed that at the 15-year follow-up, significantly higher MIH (for the affected permanent teeth) values were observed only in the asthmatics who were not taking metered-dose inhalers, in the fully adjusted models. It could be presumed that poor asthma control probably leads to more frequent episodes of oxygen deprivation, enhancing the chances of ameloblasts injury, and consequently enamel defects.

It is of prime importance to emphasize that the majority of the appraised studies in this systematic review were cross-sectional or case-control, and in these designs, causality cannot be established, being unable to strongly affirm whether teeth defects were a consequence of asthma. Moreover, many studies[5,22,23,25–29,31,32,34,37,38] employed questionnaires or interviews to obtain information regarding children's medical or drugs history and asthma diagnosis in

early childhood. Considering the odontogenesis period, any health problem in the early years of life, until about 4–5 years is capable of modulating ameloblasts activity and prejudicing amelogenesis. Although caregivers are attentive to their children in the first years of life, relying upon their memories could lead to potential bias. Thus, further prospective longitudinal studies with a powerful sample size, including stratification by age and primary/permanent dentition, as well as including medical reports (major tools in the diagnosis of asthma assessment) would be of great value; especially because the target risk factors would precede the enamel defects when identified. Still, a single standard criterion for enamel defects would also be stimulated, for better comparing and interpreting the results. Of interest, most appraised studies employed the EAPD or the DDE index, which are different.

In four studies,[27,28,31,33] asthma was not evaluated alone, but together with bronchitis. Therefore, these data need to be interpreted with caution, as the effect could have been diminished if asthma were assessed alone. While asthma is a chronic inflammatory disease,[30] which can be controlled but not cured,[47] bronchitis/bronchiolitis during childhood is acute and associated with infections.[30] Both diseases are linked to episodes of oxygen deprivation, which can have detrimental effects on amelogenesis, leading to hypomineralization.[23]

A better understanding of the issue addressed in the present systematic review revealed an association between asthma and enamel defects in young permanent teeth. In addition, asthma has been closely linked to MIH, a specific dental enamel imperfection of rising global interest in recent years.

From a critical point of view, teeth cells are part of the human body and could not be considered separate. It would be plausible that childhood systemic conditions affecting growing cells may certainly impact enamel-forming cells. The connection between teeth defects, especially MIH, and asthma experience in early childhood underscores the significance of a multidisciplinary approach, bringing together professionals with different expertise. Pediatricians would pay attention that children with asthma could be more likely to have enamel defects in permanent teeth, warning parents about the necessity for greater oral hygiene for their children. This is because hypomineralization can lead to posteruptive enamel breakdown and atypical cavities, favoring biofilm accumulation and carious lesions development. In the same way, pedodontists would also be aware of their patient's general health, as asthmatic children, especially the poorly controlled, are prone to develop enamel defects, particularly in first permanent molars, which should be cautiously monitored, soon after the eruption, avoiding early loss of tooth structure and future restoration and rehabilitation. Besides, the interrelationship between these professionals would contribute to the early diagnosis and assertive strategies diminishing the chances of future teeth sensitivity and discomfort, impacting oral health and even the quality of life.

To top it all, systematic reviews are of prime importance appraising critically the published papers, favoring the clinical practice based on scientific evidence.

5 | CONCLUSION

It can be concluded that asthma is closely associated with enamel defects in young permanent teeth. However, as most of the papers appraised were of cross-sectional or case-control design, further well-designed clinical investigations with a prospective design are welcome to reinforce our findings.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Karina F. Rizzardi: Conceptualization, Data curation, Formal analysis, Methodology, Visualization, Writing – original draft. Elora da Silva Toledo: Writing – original draft. Lucio F. C. Ferraz, Michelle Darrieux, and Raquel Girardello: Writing – review and editing. Fernando A. de Lima Marson: Formal analysis, Writing – review and editing. Thais M. Parisotto: Conceptualization, Data curation, Formal analysis, Methodology, Visualization, Supervision, Writing – review and editing.

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REFERENCES

1. Kühnisch J, Lauenstein A, Pitchika V, et al. Was molar incisor hypomineralisation (MIH) present in archaeological case series? *Clin Oral Investig*. 2016;20:2387-2393.
2. Feltrin-Souza J, Jeremias F, Alaluusua S, et al. The effect of amoxicillin on dental enamel development in vivo. *Braz Oral Res*. 2020;34:1-9.
3. Nanci A. Enamel: composition, development, and structure. In: Nanci A, ed. *Ten Cate's Oral Histology, Development, Structure, and Function*. Missouri: Elsevier; 2018:118-1155.
4. Guedes-Pinto AC. *Odontopediatria*. Vol 4. Rio de Janeiro: Santos; 2016:836.
5. Pitiphat W, Luangchaichaweng S, Pungchanchaikul P, Angwaravong O, Chansamak N. Factors associated with molar incisor hypomineralization in Thai children. *Eur J Oral Sci*. 2014;122:265-270.
6. Koch MJ, Buhrer R, Pioch T, Schärer K. Enamel hypoplasia of primary teeth in chronic renal failure. *Pediatr Nephrol*. 1999;13:68-72.
7. Alaluusua S. Aetiology of molar-incisor hypomineralisation: a systematic review. *Eur Arch Paediatr Dent*. 2010;11:53-58.
8. Van Amerongen WE, Kreulen CM. Cheese molars: a pilot study of the etiology of hypocalcifications in first permanent molars. *ASDC J Dent Child*. 1995;62:266-269.
9. Sundfeld D, da Silva L, Kluppel OJ, et al. Molar incisor hypomineralization: etiology, clinical aspects, and a restorative treatment case report. *Oper Dent*. 2020;45:343-351.
10. Fejerskov O, Nyvad B, Kidd E, eds. *Dental Caries: The Disease and Its Clinical Management*. New York: John Wiley & Sons; 2015:480.
11. de Oliveira DC, Favretto CO, Cunha RF. Molar incisor hypomineralization: considerations about treatment in a controlled longitudinal case. *J Indian Soc Pedod Prev Dent*. 2015;33:152-155.
12. Da Costa-Silva CM, Ambrosano GM, Jeremias F, De Souza JF, Mialhe FL. Increase in severity of molar-incisor hypomineralization

and its relationship with the color of enamel opacity: a prospective cohort study. *Int J Paediatr Dent*. 2011;21:333-341.
13. Ghanim A, Manton D, Marino R, Morgan M, Bailey D. Prevalence of demarcated hypomineralisation defects in second primary molars in Iraqi children. *Int J Paediatr Dent*. 2013;23:48-55.
14. Lygidakis NA, Dimou G, Marinou D. Molar-incisor-hypomineralisation (MIH). A retrospective clinical study in Greek children. II Possible medical aetiological factors. *Eur Arch Paediatr Dent*. 2008;9:207-217.
15. Jalevik B, Noren JG, Klingberg G, Barregård L. Etiologic factors influencing the prevalence of demarcated opacities in permanent first molars in a group of Swedish children. *Eur J Oral Sci*. 2001;109:230-234.
16. Smits HH, Hiemstra PS, Prazeres da Costa C, et al. Microbes and asthma: opportunities for intervention. *J Allergy Clin Immunol*. 2016;137:690-697.
17. Indiani CMDSP, Rizzardi KF, Castelo PM, Ferraz LFC, Darrieux M, Parisotto TM. Childhood obesity and Firmicutes/Bacteroidetes ratio in the gut microbiota: a systematic review. *Child Obes*. 2018;14:501-509.
18. Visweswar VK, Amarlal D, Veerabahu R. Prevalence of developmental defects of enamel in children and adolescents with asthma: a cross-sectional study. *Indian J Dent Res*. 2012;23:697-698.
19. Elfrink ME, Moll HA, Kiefte-de Jong JC, El Marroun H, Jaddoe VW, Hofman A, Veerkamp JS. Is maternal use of medicines during pregnancy associated with deciduous molar hypomineralisation in the offspring? A prospective, population-based study. *Drug Saf*. 2013;36:627-633.
20. Mastora A, Vadiakas G, Agouropoulos A, Gartagani-Panagiotopoulou P, Engesaeth VG. Developmental defects of enamel in first permanent molars associated with use of asthma drugs in preschool-aged children: a retrospective case-control study. *Eur Arch Paediatr Dent*. 2017;18:105-111.
21. Wogelius P, Viuff JH, Haubek D. Use of asthma drugs and prevalence of molar incisor hypomineralization. *Int J Paediat Dent*. 2020;30:734-740.
22. Ford D, Seow WK, Kazoullis S, Holcombe T, Newman B. A controlled study of risk factors for enamel hypoplasia in the permanent dentition. *Pediatr Dent*. 2009;31:382-388.
23. Guergolette RP, Dezan CC, Frossard WTG, Ferreira FBDA, Cerci Neto A, Fernandes KBP. Prevalence of developmental defects of enamel in children and adolescents with asthma. *J Bras Pneumol*. 2009;35:295-300.
24. Ferrazzano GF, Sangianantoni G, Cantile T, Amato I, Ingenito A, Noschese P. Dental health in asthmatic children: a South Italy study. *ASDC J Dent Child*. 2012;79:170-175.
25. Durmus B, Abbasoglu Z, Kargul B. Possible medical aetiological factors and characteristics of molar incisor hypomineralisation in a group of Turkish children. *Acta Stomatol Croat*. 2013;47:297-305.
26. Allazzam SM, Alaki SM, El Meligy OAS. Molar incisor hypomineralization, prevalence, and etiology. *Int J Dent*. 2014;2014:234508.
27. Tourino LF, Corrêa-Faria P, Ferreira RC, Bendo CB, Zarzar PM, Vale MP. Association between molar incisor hypomineralization in schoolchildren and both prenatal and postnatal factors: a population-based study. *PLOS One*. 2016;11:e0156332.
28. Hernandez M, Boj J, Espasa E, Planells P, Peretz B. Molar-incisor hypomineralization: positive correlation with atopic dermatitis and food allergies. *J Clin Pediatr Dent*. 2018;42(5):344-348.
29. Koruyucu M, Özel S, Tuna EB. Prevalence and etiology of molar-incisor hypomineralization (MIH) in the city of Istanbul. *J Dent Sci*. 2018;13:318-328.
30. Rezende G, dos Santos NML, Stein C, Hilgert JB, Faustino-Silva DD. Asthma and oral changes in children: associated factors in a community of southern Brazil. *Int J Paediatr Dent*. 2019;29:456-463.
31. Kılınç G, Çetin M, Köse B, Ellidokuz H. Prevalence, aetiology, and treatment of molar incisor hypomineralization in children living in Izmir City (Turkey). *Int J Paediatr Dent*. 2019;29:775-782.
32. Lima LRS, Pereira AS, de Moura MS, et al. Pre-term birth and asthma is associated with hypomineralized second primary molars in preschoolers: a population-based study. *Int J Paediatr Dent*. 2020;30:193-201.
33. Hernandez M, Mendioroz J. Molar-incisor hypomineralisation and allergic march. *Acta Stomatol Croat*. 2020;54:2130-2135.
34. Neto MBC, Silva-Souza KPD, Maranhão VF, Botelho KVG, Helmer MV. Enamel defects in deciduous dentition and their association with the occurrence of adverse effects from pregnancy to early childhood. *Oral Health Prev Dent*. 2020;18:741-746.
35. Wogelius P, Haubek D, Nechifor A, Nørgaard M, Tvedebrink T, Poulsen S. Association between use of asthma drugs and prevalence of demarcated opacities in permanent first molars in 6-to-8-year-old Danish children. *Community Dent Oral Epidemiol*. 2010;38:145-151.
36. Loli D, Costacurta M, Maturo P, Docimo R. Correlation between aerosol therapy in early childhood and molar incisor hypomineralisation. *Eur J Paediatr Dent*. 2015;16:73-77.
37. Flexeder C, Hassan LK, Standl M, Schulz H, Kühnisch J. Is there an association between asthma and dental caries and molar incisor hypomineralisation? *Caries Res*. 2020;5:86-94.
38. Jälevik B, Norén JG, Klingberg G, Barregård L. Etiologic factors influencing the prevalence of demarcated opacities in permanent first molars in a group of Swedish children. *Eur J Oral Sci*. 2001;109:230-234.
39. Kühnisch J, Mach D, Thiering E, et al. Respiratory diseases are associated with molar-incisor hypomineralizations. *Swiss Dent J*. 2014;124:286-293.
40. de Lima MD, Andrade MJ, Dantas-Neta NB, et al. Epidemiologic study of molar-incisor hypomineralization in schoolchildren in North-eastern Brazil. *Pediatr Dent*. 2015;37:513-519.
41. Farah RA, Monk BC, Swain MV, Drummond BK. Protein content of molar–incisor hypomineralisation enamel. *J Dent*. 2010;38:591-596.
42. Simmer JP, Fincham AG. Molecular mechanisms of dental enamel formation. *Crit Rev Oral Biol Med*. 1995;6:84-108.
43. Sui W, Boyd C, Wright JT. Altered pH regulation during enamel development in the cystic fibrosis mouse incisor. *J Dent Res*. 2003;82:388-392.
44. Subbarao P, Mandhane PJ, Sears MR. Asthma: epidemiology, etiology and risk factors. *CMAJ*. 2009;181:E181-E190.
45. Lygidakis Na, Wong F, Jalevik B, Vierrou AM, Alaluusua S, Espelid I. Best clinical practice guidance for clinicians dealing with children presenting with molar-incisor-hypomineralisation (MIH): an EAPD policy document. *Eur Arch Paediatr Dent*. 2010;11:75-81.
46. Rehman Q, Lane NE. Effect of glucocorticoids on bone density. *Med Pediatr*. 2003;41:212-216.
47. Getch YQ, Neuharth-Pritchett S. Teacher characteristics and knowledge of asthma. *Public Health Nurs*. 2009;26:124-133.

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4. CAPÍTULO II - Artigo submetido ao periódico "*Translational Pediatrics"*

Dentes com defeitos em sua estrutura, como os acometidos pela Hipomineralização Molar-Incisivo (HMI), são mais susceptíveis ao desenvolvimento de lesões cariosas. A cárie é uma doença complexa e multifatorial altamente prevalente na infância. Este capítulo da tese tem o objetivo de avaliar a relação entre os estágios de HMI e a presença de bactérias cariogênicas em crianças. No estudo transverso, 566 escolares (6-8 anos), regularmente matriculados em Bragança Paulista-SP, foram submetidos a avaliação de presença de HMI e cárie, por meio de exames clínicos utilizando-se critério da Associação Europeia de Odontopediatria para HMI e o critério da Organização Mundial da Saúde modificado para cárie. Quarenta escolares foram distribuídos igualmente em 4 grupos: G1- Primeiros molares permanentes saudáveis; G2 - HMI leve com opacidade branca e livre de cárie, G3 - HMI leve com opacidade amarela e livre de cárie, G4 - HMI grave com opacidade branca/amarela e presença de cárie. O biofilme dental foi avaliado para quantificação de *S. mutans* e *Lactobacillus* spp. por PCR em tempo real, e a ingestão de açúcar pelo Questionário Alimentar do Dia Anterior. Os dados do estudo transverso foram analisados por meio de análise de variância seguida do teste de Tukey, Qui-quadrado e regressão de Poisson. As crianças sem HMI e sem cárie (G1) e as com HMI leve (G2) comportam-se de forma semelhante quando comparadas com G3 e G4. Além disso, o teste de Tukey evidenciou que HMI grave juntamente com cárie (G4) apresentou maior proporção de *S. mutans* e *Lactobacillus* spp. quando comparado com G2 ($p<0,05$). Curiosamente, aumentos na proporção de *Lactobacillus* spp. foram associados a um aumento da severidade da HMI (RR:7; $p=0,03$). As conclusões do estudo transversal revelaram que os níveis de *Lactobacillus* spp. foram influenciados por diferentes graus de HMI e a presença de cárie. É importante salientar que identificar HMI precocemente, como logo após a erupção desses elementos na cavidade bucal, conforme realizado no presente estudo, possibilita pontuar quem está sob risco, favorecendo o direcionamento efetivo dos tratamentos preventivos.

RELATIONSHIP BETWEEN CARIOGENIC BACTERIA AND MOLAR INCISOR HYPOMINERALIZATION IN THE CHILDHOOD

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Abstract

Teeth with defects in their structure, such as the ones affected by Molar-Incisor Hypomineralization (MIH), are more susceptible to the development of carious lesions. Caries is a complex and multifactorial disease highly prevalent in childhood. The present research aimed at evaluating the relationship between the presence of MIH, dental caries, and cariogenic bacteria in children. Forty scholars were equally assigned into 4 groups: healthy (G1); mild MIH (G2 and G3) and severe MIH + caries (G4). Dental biofilm was assessed for quantification of *Streptococcus mutans* and *Lactobacillus spp.* by real-time – PCR. Sugar intake was estimated by the Previous Day Food Questionnaire. Children without MIH and free of caries (G1) and the ones with mild MIH characterized by white opacities (G2) behave similarly when compared with the ones with yellow opacities (G3) or with severe MIH + caries (G4). The posthoc Tukey test evidenced that G4 had greater levels of *S. mutans and Lactobacillus spp*. when compared with G2 ($p<0.05$); however, the control group did not diverge from the others considering *S. mutans* ($p>0.05$). Interestingly, increases in Lactobacillus spp. enhance the severity of MIH (RR:7; $p=0.03$). In conclusion, *Lactobacillus spp.* were influenced by different degrees of MIH and the presence of caries.

Keywords: Dental caries. Susceptibility to Dental Caries. Tooth Demineralization.

Brief Reports

Introduction

Molar incisor hypomineralization (MIH) is a qualitative developmental defect of the enamel of systemic origin, affecting obligatorily at least one of the first permanent molars, involving or not the incisors (1). The process of enamel formation is highly sensitive and genetically controlled, but polymorphisms in involved genes could occur. The permanent first molars and incisors formation begins in the final period of pregnancy and ends around three to five years of age (2,3). The identification of the factors that can affect the enamel-producing cells in this period is challenging and the exact etiology of MIH is not completely understood. A recent paper suggests an association with oxygen deprivation pathologies in young children, such as asthma (4).

The tooth affected by MIH may present a mild degree, characterized by changes in the color of the enamel (white to yellow/brownish), or severe with post-eruptive enamel breakdown. The defective enamel is extremely susceptible to fracture due to masticatory forces, and provides a perfect environment for biofilm accumulation and carious process development. In addition, these teeth could be highly sensitive (5).

The relationship between dental caries and MIH has already been evidenced and a recent study showed that the presence of MIH was associated with 6.15 times higher caries prevalence in the first permanent molars (6).

In the scientific literature studies involving MIH, oral microbiota, specially the classical acidogenics and acidurics ones related to caries (*S. mutans* and *Lactobacillus* spp.) and molecular biology techniques are extremely scarce (7,8). Furthermore, MIH is a topic widely discussed nowadays, being about 819 articles published in PubMed in the last 5 years. A 2018 systematic review revealed that MIH is expressively prevalent worldwide (10,7 – 15,3%) (9). The present research aimed to assess the relationship between the presence of MIH, caries, and cariogenic bacteria in children.

Methods

Ethical considerations

This study was approved by the Ethics Committee of the University São Francisco, USF (protocol number: 10408119.0.0000.5514). Guardians who agreed with the participation of their child in this study signed an informed positive consent form, following the Helsinki declaration.

Sampling characteristics

The present cross-sectional research comprised Brazilian children of both sexes, aged 6 to 8 years of age, attending the largest public schools in the central area of Bragança Paulista-SP, Brazil. This municipality has about 172.346 inhabitants, a human development index of 0.776, and an amount of fluoride in drinking water of 0.69 mg/L.

As part of a larger study encompassing 566 children, forty children were divided into the following groups (after caries and MIH diagnosis), according to a convenience sampling strategy: G1- Healthy first permanent molars; G2 - Mild MIH with white opacity and free of caries, G3 - Mild MIH with yellow opacity and free of caries, G4 - Severe MIH with white/yellow opacities and presence of caries (Fig. 1). The MIH groups (G2, G3 and G4) account for 166 affected teeth with different degrees of severity (75,3% molars and 24,7% incisors): G2 = 34 molars + 7 incisors, G3 = 41 molars + 21 incisors and G4 = 50 molars +13 incisors.

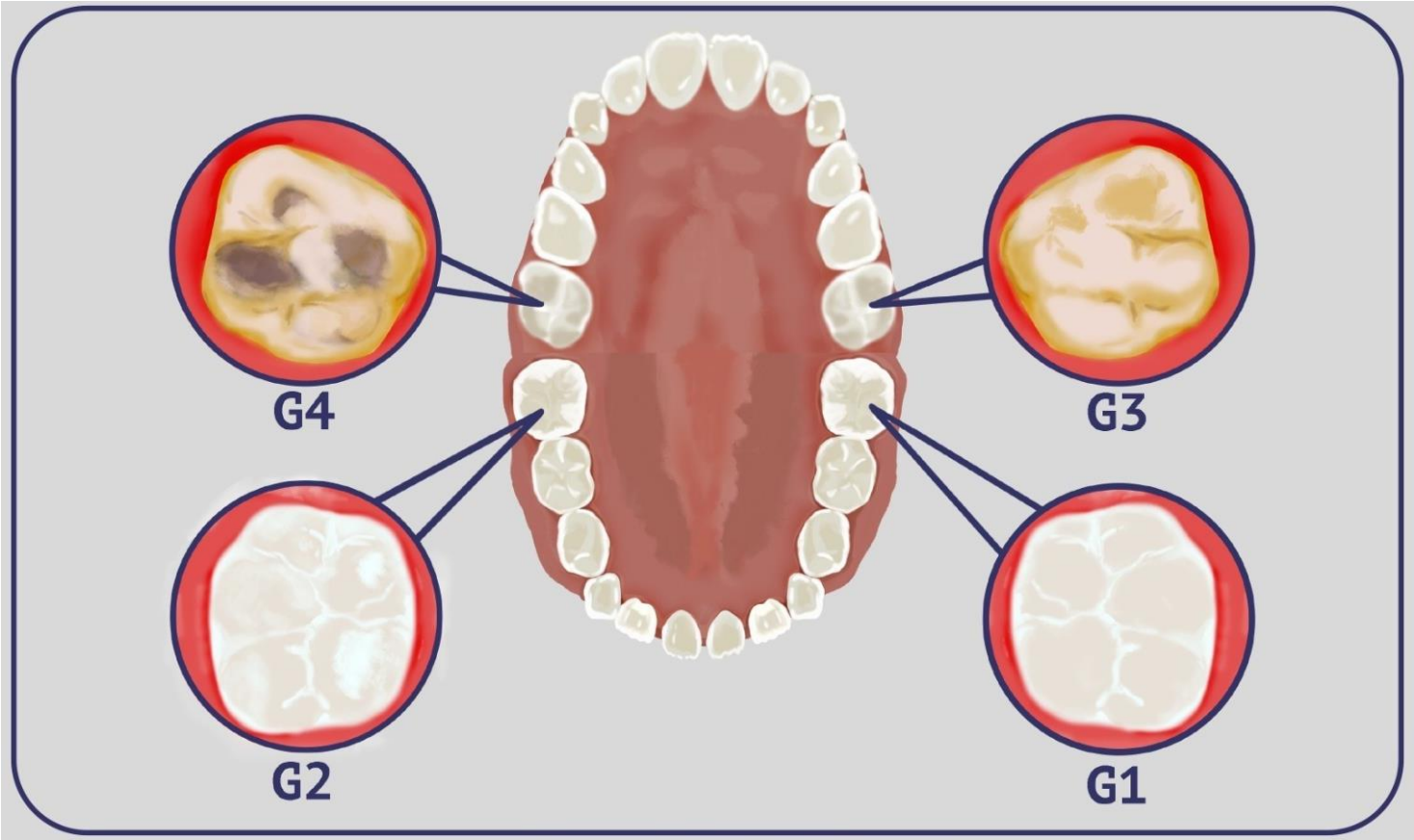

Figure legend: Sound enamel – G1, Mild MIH with white opacity and free of caries - G2, Mild MIH with yellow opacity and free of caries - G3, Severe MIH with white/yellow opacities and presence of caries - G4

FIGURE 1. Degree of severity of molar incisor hypomineralization *versus* sound enamel.

Enrolled scholars were from similar socioeconomic backgrounds (low to mid) at the schoolchildren and the brushing was performed with fluoridated toothpaste. Children taking antibiotics or pre/probiotics at the time of the biofilm collection or using these medications in the 30 days preceding the biofilm collection were excluded. Also, the ones with special needs were dismissed.

Assessment of the daily frequency of sugar exposure

The daily frequency of sugar exposure was assessed using the "Food Questionnaire of the Previous Day - QUADA-3" (10). According to illustrative images, children were asked to show the food groups eaten in all meals in the previous day (at school or at home), increasing the fidelity

of the data collected. Based on QUADA-3, the frequency of daily sugar exposure in the liquid and solid forms was estimated.

Assessment of health conditions

The evaluation of the child's history of peri and postnatal conditions were collected using a structured and self-administered questionnaire to parents or guardians. The following questions were asked: “Do you have problems in the last trimester of pregnancy, considering fever/urinary infection/ antibiotics usage?”, “What were the baby`s perinatal conditions considering delivery mode/respiratory difficulties/incubator necessity/weight/ preterm birth?” and “Do your child have pneumonia, asthma, sinusitis, rhinitis, or use antibiotics until de second year of life?”. The questions were settled based on the study of Lima (11).

Assessing early childhood caries and MIH through clinical examination

The children had their teeth cleaned and dried with gauze. The diagnosis of MIH and dental caries was carried out using visual inspection, under a headset light, with a mirror and a ball-ended dental probe. The criteria of the European Association of Pediatric Dentistry – EAPD (12) and World Health Organization – WHO (13) criteria modified by the inclusion of active white spot lesions were used. Thus, both cavitated and non-cavitated lesions were diagnosed as caries in this study. The scores for diagnosing MIH are illustrated in BOX 1. The clinical examinations were performed by a dentist and calibrated by a gold-standard examiner. At the beginning of the study, after the examiner had received all theoretical and practical guidelines on the criteria to be used, about 10 children were re-examined with a time interval of at least one week between the examinations. The calculated inter-examiner Kappa values were: 0.82 for MIH and 0.99 for dental caries.

BOX 1. Criteria for molar incisor hypomineralization (MIH) assessment according to the European Association of Pediatric Dentistry

Code	Criteria
0	Enamel defect free
1	White⁄creamy demarcated opacities, no PEB
1a	White⁄creamy demarcated opacities, with PEB
2	Yellow/brown, demarcated opacities, no PEB
2a	Yellow/brown, demarcated opacities, with PEB
3	Atypical restoration
4	Missing because of MIH
5	Partially erupted (i.e., less than one-third of the crown) with evidence of MIH
6	Unerupted⁄partially erupted with no evidence of MIH
7	Diffuse opacities (not MIH)
8	Hypoplasia (not MIH)
9	Combined lesion (diffuse opacity⁄hypoplasia with MIH)
10	Demarcated opacities in incisors only

PEB: Post-eruptive Enamel Breakdown

Biofilm collection

Biofilm samples were collected from all surfaces of the affected first permanent molars in the experimental groups (G2, G3, and G4) and all surfaces of one healthy first molar in the control group (G1). The samples were placed into microcentrifuge tubes, which were kept inside an icebox during the collection period. In the Laboratory of Microbiology of the University São Francisco, the collected biofilm was frozen at -80°C until DNA extraction and RT-PCR analysis to quantify the species: *S. mutans*, *Lactobacillus* spp. and the Firmicutes phylum.

Analysis through real-time polymerase chain reaction (RT-PCR)

DNA extraction from dental biofilm samples was performed using the Lucigen/Epicentre kit (MasterPure™ Complete DNA and RNA Purification Kit, Cat. #MC85200) and the DNA concentration was estimated in the Biodrop equipment (Biodrop µLite Spectrophotometer, Biochrom US Inc., Holliston, MA, USA). Concisely, the biofilm was resuspended in 300 µl of a *Tissue and Cell Lysis Solution* (containing proteinase K) and incubated at 65°C for 15 minutes. After placing samples on ice for 5 min, 150 µL of an *MPC protein precipitation reagent* was

inserted and centrifugation was performed (10 min, 10,000 x g, 4°C). The supernatant was transferred to a clean microcentrifuge tube and the pellet discarded. Isopropanol (500 µL) was added to the supernatant and mixed gently by inversion. After centrifugation (10 min, 10,000 x g, 4°C), total nucleic acids were obtained, rinsed twice with 70% ethanol, and resuspended in TE Buffer (35 µL). The DNA was used for identifying the species: SM, LB, and Firmicutes phylum through real-time PCR.

Real-time assays were performed on the 7300 Real-Time System, (Applied Biosystems, Foster City, CA, USA), using the SYBR Green Power up (Thermo Fisher Scientific, Carlsbad, CA, USA) reagent. Primer sequences, genes, and amplicons are described in BOX 2 above:

BOX 2. Phylum/species, target genes and primers for identification and quantification of bacteria by SYBR Green RT-PCR.

Phylum/species	Target genes	Amplicon size	Primers
Firmicutes[14]	16S rRNA	126	FP: GGAGYATGTGGTTTAATTCGAAGCA RP: AGCTGACGACAACCATGCAC
Streptococcus mutan[15]	gtfB gene	114	FP: GCCTACAGCTCAGAGATGCTATTCT RP: GCCATACACCACTCATGAATTGA
Lactobacillus spp.[16]	16S rRNA	126	FP: GAGGCAGCAGTAGGGAATCTTC RP:GGCCAGTTACTACCTCTATCCTTCTTC

A total of 1.5µl of the DNA extracted from biofilm samples was used for each assay, together with 5 µL of SYBR Green Power up (Thermo Fisher Scientific), 2.9 µL of H_2O, 0.3 µL of forwarding primer, and 0.3 µL of the reverse primer.

The cycling for the detection of SM, LB, and Firmicutes phylum included 50°C for 2 min, 95°C for 10 min, and 40 cycles of 95°C for 15s followed by 60° for 1min.

Standard curves were performed to determine the absolute target quantity in samples, using the following species: SM (UA159 - ATCC 70061) LB (*Lactobacillus casei* - clinical strain previously identified at the Laboratory of Clinical and Molecular Microbiology – USF), and Firmicutes phylum (*Clostridium perfringens* - ATCC 13124) which were also used as positive controls. DNA samples of control strains were serially diluted ten-fold (five dilution points; 10^1 – 10^5 ηg DNA/µl) to generate standard curves for determining the absolute target quantity in samples.

Considering the standard curves, the software (Sequence Detection Software version 1.3.1, Applied Biosystems, Foster City, CA, USA) interpolates the absolute quantity of the target in the test samples.

The critical threshold cycle was the one in which the detectable fluorescence was above the background (standard threshold: 0.200). Duplicates were performed in all RT-PCR assays: standards and DNA samples. Bacteria levels were expressed by ηg DNA/ µl.

Statistical Approach

Data were statistically analyzed using the SPSS package for Windows, version 21.0 (SPSS, Inc., Chicago, IL, USA). Gaussian distribution was tested using the Shapiro-Wilk test and Quantile-quantile-plot (QQ-plot) analysis. Levene`s test was used to prove homogeneity of variance. SM and LB were the dependent variables and were adjusted by dividing them by Firmicutes phylum levels**.**

The exploratory statistics consisted of medians and interquartile ranges. Data were transformed using normal logarithmic expression to adhere to the Analysis of Variance premises. The one-way analysis of variance followed by the Tukey test was used considering a 0.05 significance level. In addition, a model assessing the significant risk indicators in different stages of MIH, concerning bacterial parameters was performed (Poisson regression analysis).

Qui-squared was performed to test the association between health conditions and dental caries and different degrees of MIH.

Results and Discussion

The mean age of the schoolers included was of 88.61 months (±10.45). Boys and girls show an equivalent distribution inside the groups. In children with severe MIH with white/yellowish opacities and the presence of caries (G4), the mean number of decayed missing or filled teeth was 2.88 (±1.05).

To the best of our knowledge, the present investigation showed for the first time in the scientific literature that LB levels were influenced by different degrees of MIH and caries (Table 1).

Table 1: *S. mutans (SM)* and *Lactobacillus* spp.*(LB) levels* according to different degrees of molar incisor hypomineralization (MIH).

	SM (ηg DNA/ µl)	**LB (ηg DNA/ µl)**
G1- Healthy first permanent molars	0.038 (0.034) ab	0.004 (0.003) a
G2 - Mild MIH with white opacity and free of caries	0.028 (0.013) b	0.002 (0.001) a
G3 - Mild MIH with yellow opacity and free of caries	0.057 (0.038) ab	0.012 (0.010) b
G4 - Severe MIH with white/yellow opacities and presence of caries	0.095 (0.084) a	0.016 (0.008) b
ANOVA p-value	0.023*	0.000*

*Statistical analyses were performed with a sample of 40 cases, 10 per group. Data were expressed in the median (interquartile range). Data were transformed using normal logarithmic expressions. SM and LB were adjusted by dividing them by Firmicutes phylum levels. Different lower letters represent statistically significant differences according to groups (between lines within the same column). *Statistically significant at p<0.05.*

Children with healthy first permanent molars (G1) and the ones with mild MIH characterized by white opacity (G2) behave similarly when compared with mild MIH characterized by yellow opacity (G3) and severe MIH with white/yellow opacities and presence of caries (G4). Besides, the posthoc Tukey test evidenced that yellow opacity (G3) or severe MIH together with caries (G4) had greater LB when compared with G2 and G1 ($p<0.05$) (Table 1). White opacities in the first permanent molars affected by MIH characterize the minor degree of the defect. This way, is plausible to expect the enamel with closer characteristics to the sound enamel. On the other hand, the yellow opacity could be suggested as a step forward in the severity scale, being more porous in comparison with the white ones, with worse prism organization and more prone to acid attacks (17). This way, yellow areas may be about similar to a stricter stage, involving fractures and dental caries. Of interest, the niche retentiveness is important for LB accumulation (18). In the case of SM, it does not need a rough surface to adhere, probably explaining why the control group did not diverge from the others considering this microbe ($p>0.05$).

Interestingly, in our investigation, we found that every one-unit increase in LB enhances by 7 times the severity of MIH (RR:7; $p=0.03$) (Table 2). LB are Gram-positive rods, acidogenic and aciduric anaerobes, and although they are secondary colonizers in pre-existing carious lesions (because they need a retentive niche, such as the rough yellow opacities, rapidly turning into enamel breakdown) they are of prime importance in the progression of these lesions (19). Their capacity of organic acid production by dietary sugar fermentation, promotes enamel dissolution and

degradation of the dentinal organic tissues, favoring its penetration in dentinal tubules. This penetration is enhanced by the wide tubules of young teeth, together with the hypomineralized dental tissues, which is the case of MIH (20).

So, it should be reinforced the role played by te microbiota in teeth affected by MIH, favoring their hypersensitivity by penetrating defective enamel and reaching sensitive tissues. Furthermore, when the first permanent molars appear in the oral cavity (around 6 years of age) underneath the occlusal plane, without contact with the antagonist's teeth during mastication (Fig 1), the biofilm accumulation is favored, together with caries susceptibility. Consequently, the presence of MIH could potentialize this process.

Importantly, the performance of clinical procedures is hampered by the great sensitivity of teeth affected by MIH due to their chronic pulp inflammation (20), prejudicing the oral health-related quality of life. In this respect, and reinforcing the role played by the microbiota in teeth affected by MIH, it was already suggested that oral bacteria penetrate the hypomineralized enamel reaching the dentin microtubules, favoring the hypersensitivity of these teeth.

When the first permanent molar appears in the oral cavity, around 6 years of age, underneath the occlusal plane without contact with the antagonist's teeth during mastication (Fig 1), the biofilm accumulation is favored, in the same way as caries susceptibility. Consequently, the presence of MIH could potentialize this process.

Table 2. Bacteria indicators in children with molar incisor hypomineralization (MIH).

	Different degrees of MIH (severity)	
Parameters	**Rate Ratio (95% CI)**	**p-value**
Lactobacillus spp. (%)	7.706 (1.153 - 51.518)	0.035*
Streptococcus mutans (%)	1.056 (0.983- 1.134)	0.134

*Poisson Regression Model; Main outcome: degress of MIH; *Statistically significant at p<0.05. CI: confidence interval; n=40; Omnibus Test: likelihood Ratio Chi-Square=4.887. The rate ratio was considered as a measure of effect size: 1.22 (small); 1.86 (medium); 3.00 (large). SM and LB were adjusted by dividing them by Firmicutes phylum levels* (ηg DNA/ µl).

In the present study, systemic diseases occurring during the last trimester of pregnancy, in the perinatal period, and in post-natal (0 to 2 years) were more frequent in MIH-affected teeth (G2, G3, and G4 -Table 3). Remarkably, during these periods occurs the genesis of the first permanent molars and incisors, precisely the dental teeth affected by MIH. These findings reinforce the production of hypomineralized enamel with concomitant medical conditions (4), as dental tissues could not be separated from the rest of the body. The linkage between MIH and systemic childhood diseases supports the importance of a multidisciplinary and holistic approach favoring early diagnosis. The identification of MIH, soon after the eruption of the first permanent molars in the oral cavity, as performed in the present research, enables assertive strategies for the affected children, avoiding complex and expensive rehabilitation, which are challenging during childhood and probably impacts the future quality of life.

Table 3: Health conditions according to caries and different degrees of molar incisor hypomineralization (MIH).

		G1	***G2***	***G3***	***G4***
Last trimester of pregnancy	*Fever/ infection (Yes)*	0	0	1 (10%)	0
	Urinary infection (Yes)	0	0	3 (30%)	1 (10%)
	Antibiotic (Yes)	0	1 (10%)	3 (30%)	1 (10%)
Perinatal conditions	*Hypoxia (Yes)*	0	2 (20%)	0	0
	Delivery (C-section)	6 (60%)	5 (50%)	5 (50%)	4 (40%)
	Dyspnea (Yes)	0	1 (10%)	0	1 (10%)
	Incubator (Yes)	0	2 (20%)	0	2 (20%)
	Birth (Premature)	2 (20%)	3 (30%)	0	1 (10%)
	Birth weight (< 2.5 kg)	2 (20%)	3 (30%)	3 (30%)	3 (30%)
Post-natal conditions until 2 years	*Pneumonia (Yes)*	1 (10%)	0	1 (10%)	2 (20%)
	Asthma (Yes)	0	1 (10%)	0	0
	Sinusitis/ Rhinitis (Yes)	0	1 (10%)	2 (20%)	1 (10%)
	Fever (Yes)	2 (20%)	6 (60%)	7 (70%)	6 (60%)
	Antibiotics (Yes)	1 (10%)	4 (40%)	6 (60%)	6 (60%)
Eating Behavior	*Sugar intake (>3 times/day)*	2 (20%)	5 (50%)	6 (60%)	7 (70%)

Statistical analyses were performed with a sample of 40 cases, 10 per group. The p-value of Qui-squared was suppressed due to no significant association.

Despite some limitations such as the narrow sample size and the popular methodology, this pioneering study warns of the importance of the development of well-designed future studies exploring the microbiota and MIH, particularly using robust methodologies such as genome sequencing. Curiously, there is only one paper available in the scientific literature in this respect (8), proposing an association of MIH with periodontopathogenic bacteria; but without considering different degrees of MIH (8).

Conclusion

In conclusion, LB were influenced by different degrees of MIH and the presence of caries.

Declarations

Ethical Statement

Confirm that the authors are accountable for all aspects of the work (if applied, including full data access, integrity of the data and the accuracy of the data analysis) in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the University São Francisco, USF (protocol number: 10408119.0.0000.5514) which classified it as minimal-risk research. All the patients were asked for their consent.

Consent for publication

All the children guardians were asked for their consent for publication.

Data availability

Data will be available upon request to the corresponding author.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Conceptualization: TMP; Acquisition of data: KFR and RFR; Methodology: KFR and TMP; Statistical Analysis: ETS and TMP; Funding acquisition: 'Not applicable'; Project administration: KFR and TMP; Supervision: TMP; Writing-review & editing: KFR, CLC, VMT and TMP; All authors read and approved the final manuscript.

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References

1. Weerheijm, KL. Molar incisor hypomineralization (MIH): clinical presentation, etiology, and management. Dent Update. 2004, 31,9–12.
2. Alaluusua, S. (2010). Aetiology of Molar-Incisor Hypomineralisation: A systematic review. European Archives of Paediatric Dentistry, 11(2), 53–58. doi:10.1007/bf03262713
3. Logan W, Kronfeldt R. Development of the human jaws and surrounding structures from birth to the age of fifteen years. Am J Dent Assoc 1933;20:379-427.
4. Rizzardi, KF, da Silva Toledo, E, Ferraz, LF, Darrieux, M, Girardello, R, de Lima Marson, FA, Parisotto, TM. Association between asthma and enamel defects in primary and young permanent teeth–a systematic review. Pediatric Pulmonology, 2022, 57(1), 26-37.
5. de Oliveira DC, Favretto CO, Cunha RF. Molar incisor hypomineralization: considerations about treatment in a controlled longitudinal case. J Indian Soc Pedod Prev Dent. 2015 Apr-Jun;33(2):152-5. DOI: 10.4103/0970-4388.155133.
6. Oreano, MDA, Santos, PS, Borgatto, AF, Bolan, M, Cardoso, M. Association between dental caries and molar-incisor hypomineralisation in first permanent molars: A hierarchical model. Community Dentistry and Oral Epidemiology. 2022.
7. Jeremias, F, Koruyucu, M, Küchler, EC, Bayram, M, Tuna, EB, Deeley, K, .. & Gencay, K. Genes expressed in dental enamel development are associated with molar-incisor hypomineralization. Archives of oral biology, 2013, 58(10):1434-1442. ISSN 0003-9969.
8. Hernández, M, Planells P, Martínez E, Mira A, Carda-Diéguez M. Microbiology of molar-incisor hypomineralization lesions. A pilot study. J Oral Microbiol. 2020 May 20;12(1):1766166. doi: 10.1080/20002297.2020.1766166. PMID: 32595912; PMCID: PMC7301705
9. Lopes, LB, Machado, V, Mascarenhas, P. et al. The prevalence of molar-incisor hypomineralization: a systematic review and meta-analysis. Sci Rep 2021, 11:22405. https://doi.org/10.1038/s41598-021-01541-7
10. Assis MAAD, Benedet J, Kerpel R, Vasconcelos FDAGD, Di Pietro PF, Kupek E. (2009). Validação da terceira versão do Questionário Alimentar do Dia Anterior (QUADA-3) para escolares de 6 a 11 anos. Cadernos de Saúde Pública, 25, 1816-1826.
11. Lima LRS, Pereira AS, de Moura MS, Lima CCB, Paiva SM, Moura LDFADD, de Deus Moura de Lima M. (2020). Pre-term birth and asthma is associated with hypomineralized second

primary molars in pre-schoolers: A population-based study. International Journal of Paediatric Dentistry, 30(2), 193-201.

12. Ghanim A, Silva MJ, Elfrink MEC, Lygidakis NA, Mariño RJ, Weerheijm KL, Manton DJ. Molar incisor hypomineralisation (MIH) training manual for clinical field surveys and practice. Eur Arch Paediatr Dent. 2017 Aug;18(4):225-242. doi: 10.1007/s40368-017-0293-9. Epub 2017 Jul 18. PMID: 28721667.
13. Parisotto Tm, Steiner-Oliveira C, De Souza-E-Silva Cm, Peres Rc, Rodrigues Lk, Nobre-Dos-Santos M (2012) Assessment Of Cavitated And Active Non-Cavitated Caries Lesions In 3-To 4-Year-Old Preschool Children: A Field Study. Int J Paediatr Dent. 22(2):92-9.
14. Rizzardi, KF, Indiani, CMDSP, Mattos-Graner, RDO, De Sousa, ET, Nobre-dos-Santos, M, Parisotto, TM. Firmicutes Levels in the Mouth Reflect the Gut Condition With Respect to Obesity and Early Childhood Caries. Frontiers in cellular and infection microbiology. 2021, 11, 593734.
15. Childers, N.K.; Osgood, R.C.; Hsu, K.L.; Manmontri, C.; Momeni, S.S.; Mahtani, H.K.; Cutter, G.R.; Ruby, J.D. Real-time quantitative polymerase chain reaction for enumeration of Streptococcus mutans from oral samples. European journal of oral sciences, v. 119, n. 6, p. 447-454, 2011. ISSN 1600-0722.
16. Murri, M.; Leiva, I; Gomez-Zumaquero, J.M.;, Tinahones, F.J.; Cardona, F.; Soriguer, F.; Queipo-Ortuño, M.I. Gut microbiota in children with type 1 diabetes differs from that in healthy children: a case-control study. BMC medicine, v. 11, n. 1, p. 46, 2013. ISSN 1741-7015.
17. Jalevik B, Noren JG. Enamel hypomineralization of permanent first molars: a morphological study and survey of possible aetiological factors. Int J Paediatr Dent. 2000;10:278–289. doi: 10.1046/j.1365-263x.2000.00210.x.
18. Indiani, CMDSP, Rizzardi, KF, Castelo, PM, Ferraz, LFC, Darrieux, M, Parisotto, TM. Childhood obesity and firmicutes/bacteroidetes ratio in the gut microbiota: a systematic review. Childhood obesity, 2018, 14(8), 501-509.
19. Parisotto, TM, Steiner-Oliveira, C, Duque, C, Peres, RCR, Rodrigues, LKA, Nobre-dos-Santos, M. Relationship among microbiological composition and presence of dental plaque, sugar exposure, social factors and different stages of early childhood caries. Archives of oral biology, 2010, 55(5), 365-373.

20. Fagrell, TG, Lingström, P, Olsson, S, Steiniger, F, Norén, JG. Bacterial invasion of dentinal tubules beneath apparently intact but hypomineralized enamel in molar teeth with molar incisor hypomineralization. International Journal of Paediatric Dentistry, 2008, 18(5), 333-340.

5. Conclusão

Por meio da revisão sistemática concluiu-se que asma está intimamente associada a defeitos de esmalte em dentes permanentes jovens. A partir do estudo transversal sugere-se que a proporção de *Lactobacillus* spp. foi influenciada por diferentes severidades de HMI e a presença de cárie.

É importante salientar que identificar HMI precocemente, como logo após a erupção dos primeiros molares permanentes na cavidade bucal, conforme realizado no presente estudo, possibilita pontuar quem está sob risco, favorecendo o direcionamento de tratamentos preventivos efetivos. Esse tipo de tratamento minimiza o risco de agravamento das lesões de HMI e do desenvolvimento de lesões cariosas nestes indivíduos, visto que as bactérias cariogênicas apresentaram-se em níveis mais elevados na superfície dos dentes com maiores graus de severidade de HMI e podem ter acesso favorecido até o órgão pulpar, em função da característica e maior porosidade do esmalte afetado.

REFERÊNCIAS BIBLIOGRÁFICAS

AFSHARI, E.; DEHGHAN, F.; VAKILI, M. A.; ABBASI, M. Prevalence of Molar-incisor hypomineralization in Iranian children–A systematic review and narrative synthesis. **BDJ open**, v. 8, n. 1, p. 1-6, 2022.

AIRES, C.; TABCHOURY, C. P. M.; CURY, A. D. B.; KOO, H.; CURY, J. A. Effect of sucrose concentration on dental biofilm formed in situ and on enamel demineralization. **Caries research**, v. 40, n. 1, p. 28-32, 2006.

BARSAMIAN-WUNSCH, P.; PARK, J. H., WATSON, M. R.; TINANOFF, N.; MINAH, G. E. Microbiological screening for cariogenic bacteria in children 9 to 36 months of age. **Pediatric dentistry**, v. 26, n. 3, p. 231-239, 2004.

BEZAMAT, M.; SOUZA, J. F.; SILVA, F. M. F.; Gene-environment interaction in molar-incisor hypomineralization. **Plos one,** *v*. 16, 2021.

BLAUT, M.; COLLINS, M.D.; WELLING, G.W.; DORÉ, J.; VAN LOO, J.; DE VOS, W. Molecular biological methods for studying the gut microbiota: the EU human gut floraproject. **British Journal of Nutrition,** v. 87, n. S2, p. S203-S211, 2002.

COOK, C.; LOPEZ, R. M.; Is molar incisor hypomineralisation (MIH) a new disease of the 21st century?. **Pediatric Dental Journal**, 2022.

DA COSTA-SILVA, C. M.; AMBROSANO, G. M.; JEREMIAS, F.; DE SOUZA, J. F.; MIALHE, F. L. Increase in severity of molar-incisor hypomineralization and its relationship with the colour of enamel opacity: a prospective cohort study. **International journal of paediatric dentistry**, v. 21, n. 5, p. 333-341, 2011.

DE LIMA, M. D. D. M.; ANDRADE, M. J. B.; DANTAS-NETA, N. B.; ANDRADE, N. S.; TEIXEIRA, R. J. P. B.; DE MOURA, M. S.; DE DEUS MOURA, L. D. F. A. Epidemiologic Study of Molar-incisor Hypomineralization in Schoolchildren in North-eastern Brazil. **Pediatric dentistry**, v. 37, n. 7, p. 513-519, 2015.

DE OLIVEIRA, D. C.; FAVRETTO, C. O.; CUNHA, R. F. Molar incisor hypomineralization: considerations about treatment in a controlled longitudinal case. **Journal of Indian Society of Pedodontics and Preventive Dentistry**, v. 33, n. 2, p. 152, 2015.

DE OLIVEIRA RIBAS, A., & CZLUSNIAK, G. D. Anomalias do esmalte dental: etiologia, diagnóstico e tratamento. Publicatio UEPG: Ciências Biológicas e da Saúde, v. 10, n. 1, 2004.

DIAS, A. P.; MARQUES, R. B. Prevalência de cárie dentária em primeiros molares permanentes de crianças de 6 a 12 anos de idade. **Revista Interdisciplinar**, v. 10, n. 3, p. 78-90, 2018.

DO NASCIMENTO LEITE, L.; DA SILVA DAMACENO, B.; LOPES, A. F. Consumo de alimentos ultraprocessados e exposição a telas de pré-escolares residentes em região de alta vulnerabilidade social em São Paulo, Brasil. **ABCS Health Sciences**, 2022.

DURBÁN, A.; ABELLAN, J.J.; JIMENEZ-HERNANDEZ, N.; PONCE, M.; PONCE, J.; SALA, T.; D'AURIA, G.; LATORRE, A.; MOYA, A. Assessing gut microbial diversity from feces and rectal mucosa. **Microb Ecol**., v.61, p.123-133, 2011.

FAGRELL, T. G.; LINGSTRÖM, P.; OLSSON. S.; STEINIGER, F.; NORÉN, J. G. Bacterial invasion of dentinal tubules beneath apparently intact but hypomineralized enamel in molar teeth with molar incisor hypomineralization. **International Journal of Paediatric Dentistry**, v. 18, n. 5, p. 333-340, 2008.

FAGRELL, T. G., DIETZ, W., JÄLEVIK, B., & NORÉN, J. G. Chemical, mechanical and morphological properties of hypomineralized enamel of permanent first molars. **Acta Odontologica Scandinavica**, v. 68, n. 4, p. 215-222, 2010.

FARAH RA, MONK BC, SWAIN MV, DRUMMOND BK. Protein content of molar–incisor hypomineralisation enamel. **J Dent**; v. 38, p. 591-596, 2010.

FEJERSKOV, O.; KIDD, E. **Cárie Dentária - a Doença e Seu Tratamento Clínico** - 2ª Ed., editora Santos, 2011.

FRAGELLI, C. M. B; JEREMIAS, F.; FELTRIN DE SOUZA, J.; PASCHOAL, M. A.; DE CÁSSIA LOIOLA CORDEIRO, R.; SANTOS-PINTO, L. Longitudinal Evaluation of the Structural Integrity of Teeth Affected by Molar Incisor Hypomineralisation. **Caries research**, v. 49, n. 4, p. 378-383, 2015.

FURRIE, E. A molecular revolution in the study of intestinal microflora. **Gut**, v. 55, n. 2, p. 141-143, 2006.

GAMBETTA-TESSINI, K.; MARIÑO, R.; GHANIM, A.; CALACHE, H.; MANTON, D.J. Knowledge, experience and perceptions regarding Molar-Incisor Hypomineralisation (HMI) amongst Australian and Chilean public oral health care practitioners. **BMC Oral Health**, v. 16, n. 1, p. 1-9, 2016.

GOMES, S. P. M.; ARCOVERDE, M. A. M.; KRAEMER, A.; DI CREDDO, S. C.; DI BURIASCO, A. R.; GOMES, S. M. Avaliação do índice ceo-d em pré-escolares dos centros municipais de Educação Infantil de Foz do Iguaçu-PR. **Research, Society and Development**, v. 11, n. 4, p. e12811424411-e12811424411, 2022.

GRANJA, G. L.; BERNARDINO, V. M. M.; LIMA, L. C. M.; ARAÚJO, L. J. S.; ARRUDA, M. J. A. L. L. A.; FERREIRA, F. M.; PAIVA, S. M.; GRANVILLE-GARCIA, A. F. Orofacial dysfunction, nonnutritive sucking habits, and dental caries influence malocclusion in children aged 8-10 years. **American Journal of Orthodontics and Dentofacial Orthopedics**, v. 162, n. 4, p. 502-509, 2022.

GROSSI, J. A.; CABRAL, R. N.; LEAL, S.C. Caries Experience in Children with and without Molar-Incisor Hypomineralisation: A Case-Control Study. **Caries research**, v. 51, n. 4, p. 419-424, 2017.

GUEDES-PINTO, A. C. **Odontopediatria**. 9a edição, editora Santos, 2016.

HAYASHI, H.; SAKAMOTO, M.; KITAHARA M.; BENNO Y. Molecular Analysis Of fecal microbiota in elderly individuals using 16S r DNA library and T-RFLP. **Microbiol. Immunol,** v.47(8), p.557-570, 2003.

HERNÁNDEZ, M.; PLANELLS, P.; MARTÍNEZ, E.; MIRA, A.; CARDA-DIÉGUEZ, M. Microbiology of molar–incisor hypomineralization lesions. A pilot study. **Journal of Oral Microbiology**, v. 12, n. 1, p. 1766166, 2020.

ILCZUK-RYPULA, D.; ZALEWSKA, M.; PIETRASZEWSKA, D.; DYBEK, A.; NITECKA-BUCHTA, A.; POSTEK-STEFAŃSKA, L. Prevalence and Possible Etiological Factors of Molar-Incisor Hypomineralization (MIH) in Population of Silesian Children in Poland: A Pilot Retrospective Cohort Study. **International Journal of Environmental Research and Public Health**, v. 19, n. 14, p. 8697, 2022.

JEREMIAS, F.; KORUYUCU, M.; KÜCHLER, E. C.; BAYRAM, M.; TUNA, E. B.; DEELEY, K.; PIERRI, R. A.; SOUZA, J. F.; FRAGELLI, C. M.; PASCHOAL, M. A.; GENCAY, K.; SEYMEN, F.; CAMINAGA, R.M.; DOS SANTOS-PINTO, L.; VIEIRA, A. R. Genes expressed in dental enamel development are associated with molar-incisor hypomineralization. **Archives of oral biology**, v. 58, n. 10, p. 1434-1442, 2013.

KALKANI, M.; BALMER, R. C.; HOMER, R.M.; DAY, P.F.; DUGGAL, M.S. Molar incisor hypomineralisation: experience and perceived challenges among dentists specialising in paediatric dentistry and a group of general dental practitioners in the UK. **European Archives of Paediatric Dentistry**, v. 17, n. 2, p. 81-88, 2016.

KEYES, P. H. The infectious and transmissible nature of experimental dental caries. Findings and implications. **Archives of oral biology**, v. 1, n. 4, p. 304-IN4, 1960.

KOLEVENTI, A.; SAKELLARI, D.; ARAPOSTATHIS, K. N.; KOTSANOS, N. Periodontal Impact of Preformed Metal Crowns on Permanent Molars of Children and Adolescents: A Pilot Study. **Pediatric Dentistry**, v. 40, n. 2, p. 117-121, 2018.

KÜHNISCH, J.; MACH, D.; THIERING, E.; BROCKOW, I.; HOFFMANN, U.; NEUMANN, C.; HEINRICH-WELTZIEN, R.; BAUER, C.P.; BERDEL, D.; VON BERG, A.; KOLETZKO, S.; GARCIA-GODOY, F.; HICKEL, R.; HEINRICH, J.; GINI Plus 10 Study Group. Respiratory diseases are associated with molar-incisor hypomineralizations. **Swiss dental journal**, v. 124, n. 3, p. 286-293, 2014.

KÜHNISCH, J.; LAUENSTEIN, A.; PITCHIKA, V.; MCGLYNN, G.; STASKIEWICZ, A.; HICKEL, R.; GRUPE, G. Was molar incisor hypomineralisation (HMI) present in archaeological case series? **Clinical oral investigations**, v. 20, n. 9, p. 2387-2393, 2016.

LAGO, J. D.; JEREMIAS, F.; BUSSANELI, D. G.; RESTREPO, M.; SANTOS-PINTO, L. A. M. Prevalência e incidência da hipomineralização molar incisivo em Araraquara. **Revista de Odontologia da UNESP**, v. 46, n. Especial, p. 0-0, 2018.

LI, E.; HAMM C.M.; GULATI, A.S.; SARTOR, R.B.; CHEN, H.; WU, X.; ZHANG,T.; ROHLF, F.J.; ZHU, W.; GU, C.; ROBERTSON, C.E.; PACE, N.R.; BOEDEKER, E.C.; HARPAZ, N.; YUAN, J.; WEINSTOCK, G.M.; SODERGREN, E.; FRANK, D.N. Inflammatory

bowel diseases phenotype, C. difficile and NOD2 genotype are associated with shifts in human ileum associated microbial composition. **PLoS One.**, v. 7, n.6, p.1-10, 2012.

LIN, A.; BIK, E.M.; COSTELLO, E.K.; DETHLEFSEN, L.; HAQUE, R.; RELMAN, D.A.; SINGH, U. Distinct distal gut microbiome diversity and composition in healthy children from Bangladesh and the United States. **PLoS One,** v.8, n.1, p. 1-19, 2013.

LIRA, A. D. L. S.; FONTENELE, M. K. V.; DE SOUSA, F. J.; DE SOUSA, F. D. C.; RIBEIRO, C. K. C., FERREIRA, L. E. G. The prevalence of caries in the primary dentition and associated factors in children in the city of Parnaíba-Piauí. **Revista Odontológica do Brasil Central**, v. 31, n. 90, p. 147-165, 2022.

LOPES, L. B.; MACHADO, V.; MASCARENHAS, P.; MENDES, J. J.; BOTELHO, J. The prevalence of molar-incisor hypomineralization: a systematic review and meta-analysis. **Scientific reports**, v. 11, n. 1, p. 1-20, 2021.

MATTOS-GRANER, R. D. O.; ZELANTE, F.; LINE, R. C. S. R.; MAYER, M. P. A. Association between caries prevalence and clinical, microbiological and dietary variables in 1.0 to 2.5-year-old Brazilian children. **Caries Research**, v. 32, n. 5, p. 319-323, 1998.

MELIN, L.; LUNDGREN, J.; MALMBERG. P.; NORÉN, JG.; TAUBE, F.; CORNELL, D.H. XRMA and ToF-SIMS Analysis of Normal and Hypomineralized Enamel. **Microscopy and microanalysis**, v. 21, n. 2, p. 407-421, 2015.

MITTAL, R.; CHANDAK, S.; CHANDWANI, M.; SINGH, P.; PIMPALE, J. Assessment of association between molar incisor hypomineralization and hypomineralized second primary molar. **Journal of International Society of Preventive & Community Dentistry**, v. 6, n. 1, p. 34, 2016.

MOIMAZ, S. A. S.; DOS SANTOS, L. F. P.; SALIBA, T. A.; SALIBA, N. A.; SALIBA, O. Prevalência de Cárie Dentária aos 12 anos: A importância da Fluoretação e da Tradição em Levantamentos. **Archives of Health Investigation**, v. 11, n. 1, p. 82-88, 2022.

MURATBEGOVIC, A.; MARKOVIC, N.; GANIBEGOVIC SELIMOVIC, M. Molar incisor hypomineralisation in Bosnia and Herzegovina: aetiology and clinical consequences in medium caries activity population. **European Archives of Paediatric Dentistry**, v. 8, n. 4, p. 189-194, 2007.

NISII, F.; MAZUR, M.; DE NUCCIO, C.; MARTUCCI, C.; SPUNTARELLI, M.; LABOZZETTA, S.; FRATINI, A.; SOZZI, S.; MARUOTTI, A.; VOZZA, I.; LUZZI, V.; BOSSU, M.; OTTOLENGHI, L.; POLIMENI A. Prevalence of molar incisor hypomineralization among school children in Rome, Italy. **Scientific Reports**, v. 12, n. 1, p. 1-8, 2022.

NOBRE-DOS-SANTOS, M.; MELO DOS SANTOS, L.; FRANCISCO, S. B.; CURY, J. A. Relationship among dental plaque composition, daily sugar exposure and caries in the primary dentition. **Caries research**, v. 36, n. 5, p. 347-352, 2002.

OREANO, M. D. A.; SANTOS, P. S.; BORGATTO, A. F.; BOLAN, M.; CARDOSO, M. Association between dental caries and molar-incisor hypomineralisation in first permanent molars: A hierarchical model. **Community Dentistry and Oral Epidemiology**, 2022.

OYEDELE, T.A.; FOLAYAN, M.O.; ADEKOYA-SOFOWORA, C.A.; OZIEGBE, E.O. Co-morbidities associated with molar-incsor hypomineralisation in 8 to 16 year old pupils in Ile-Ife, Nigeria. **BMC Oral Health**, v. 15, n. 1, p. 1-5, 2015.

PAES LEME, A. F.; KOO, H.; BELLATO, C. M.; BEDI, G.; CURY, J. A. The role of sucrose in cariogenic dental biofilm formation--new insight. **Journal of dental research**, v. 85, n. 10, p. 878-887, 2006.

PARISOTTO, T. M.; STEINER-OLIVEIRA, C.; SOUZA-E-SILVA, C. M.; ALMEIDA, M. E. C.; RODRIGUES, L. K.; NOBRE-DOS-SANTOS, M. A Importância da prática de alimentacão, higiene bucal e fatores sócio-economicos na prevalência da cárie precoce da infancia em pré-escolares de Itatiba-SP. **Revista Odontológica do Brasil Central**, v. 19, n. 51, 2010.

PARISOTTO, T.M.; STEINER-OLIVEIRA, C.; DUQUE, C.; PERES, R. C.; RODRIGUES, L. K.; NOBRE-DOS-SANTOS, M. Relationship among microbiological composition and presence of dental plaque, sugar exposure, social factors and different stages of early childhood caries. **Archives of oral biology**, v. 55, n. 5, p. 365-373, 2010b.

REHMAN, A.; LEPAGE, P.; NOLTE, A.; HELLMIG, S.; SCHREIBER, S.; OTT, S.J. Transcriptional activity of the dominant gut mucosal microbiota in chronic inflammatory bowel disease patients. **J Med Microbiol**., v. 59, p. 1114-1122, 2010.

REIS, P. P. G.; JORGE, R. C.; AMERICANO, G. C. A.; THIAGO PONTES, N. D. S.; PERES, A. M. A. M.; SOVIERO, V. M. Prevalence and Severity of Molar Incisor Hypomineralization in Brazilian Children. **Pediatric Dentistry,** v. 43, n. 4, p. 270-275, 2021.

REYES, M. R. T.; FATTURI, A. L.; MENEZES, J. V. N. B.; FRAIZ, F. C.; ASSUNÇÃO, L. R. D. S.; SOUZA, J. F. D. Demarcated opacity in primary teeth increases the prevalence of molar incisor hypomineralization. **Brazilian oral research**, v. 33, 2019.

REZENDE, G.; DOS SANTOS, N. M. L.; STEIN, C.; HILGERT, J. B.; FAUSTINO-SILVA, D. D. Asthma and oral changes in children: Associated factors in a community of southern Brazil. Int **J Paediatr Dent**; v. 29, p. 456-463, 2019.

RIZZARDI, K.F.; DA SILVA TOLEDO, E.; FERRAZ, L. F. C.; DARRIEUX, M.; GIRARDELLO, R.; DE LIMA MARSON, F. A.; PARISOTTO, T. M. Association between asthma and enamel defects in primary and young permanent teeth - A systematic review. **Pediatric Pulmonology**, v. 57, n. 1, p. 26-37, 2022.

SELWITZ, R. H.; ISMAIL, A. I.; PITTS, N.B. Dental caries. **The Lancet**, v. 369, n. 9555, p. 51-59, 2007.

SHEN, X.J.; RAWLS, J.F.; RANDALL, T.; BURCAL, L; MPANDE, C.N.; JENKINS, N.; JOVOV, B.; ABDO, Z.; SANDLER, R.S.; KEKU, T.O. Molecular Characterization of mucosal adherent bacteria and associations with colorectal adenomas. **Gut Microbes**., v.1, n.3, p.138-147, 2010.

SILVA, F. M. F. D.; ZHOU, Y.; VIEIRA, F. G. D. F.; CARVALHO, F. M. D.; COSTA, M. D. C.; VIEIRA, A. R. Defining the prevalence of molar incisor hypomineralization in Brazil. **Pesquisa Brasileira em Odontopediatria e Clínica Integrada**, v. 20, 2020.

SIMMER, J.P.; FINCHAM, A.G. Molecular mechanisms of dental enamel formation. **Crit Rev Oral Biol Med** v. 6, p. 84-108, 1995.

STRAUSBERG, R.L.; LEVY, S.; ROGERS, Y.H. Emerging Dna Sequencing Technologies for human genomic medicine. **Drug Discov Today**, v.13, n. 13/14, p.569-77, 2008.

SUBRAMANIAM, P.; GUPTA, T.; SHARMA, A. Prevalence of molar incisor hypomineralization in 7-9-year-old children of Bengaluru City, India. **Contemporary clinical dentistry**, v. 7, n. 1, p. 11, 2016.

SUI, W.; BOYD, C.; WRIGHT, J.T. Altered pH regulation during enamel development in the cystic fibrosis mouse incisor. **J Dent Res** v. 82, p. 388–392, 2003.

TARIQ, A.; ALAM ANSARI, M.; OWAIS ISMAIL, M.; MEMON, Z. Association of the use of bacterial cell wall synthesis Inhibitor drugs in early childhood with the Developmental Defects of Enamel. **Pakistan Journal of Medical Sciences**, v. 30, n. 2, p. 393, 2014.

TOURINO, L. F.; CORRÊA-FARIA, P.; FERREIRA, R. C.; BENDO, C. B.; ZARZAR, P. M.; VALE, M. P. Association between Molar Incisor Hypomineralization in Schoolchildren and Both Prenatal and Postnatal Factors: A Population-Based Study. **PloS one**, v. 11, n. 6, p. e0156332, 2016.

TSUZUKI, F. M.; SILVA, J. C.; ISHIZU, L.; CALAZANS, C. M.; DA SILVA, M. C.; DA ROCHA, N. B. Prevalência e severidade da cárie dentária em um município em condição de vulnerabilidade social no Estado do Paraná. **Archives Of Health Investigation**, v. 7, n. 4, 2018.

VANHÉE, T.; PONCELET, J.; CHEIKH-ALI, S.; BOTTENBERG, P. Prevalence, Caries, Dental Anxiety and Quality of Life in Children with MIH in Brussels, Belgium. **Journal of Clinical Medicine**, v. 11, n. 11, p. 3065, 2022.

VILLANUEVA-GUTIÉRREZ, T.; IRIGOYEN-CAMACHO, M. E.; CASTAÑO-SEIQUIER, A.; ZEPEDA-ZEPEDA, M. A.; SANCHEZ-PÉREZ, L.; FRECHERO, N. M. Prevalence and severity of molar–incisor hypomineralization, maternal education, and dental caries: a cross-sectional study of Mexican schoolchildren with low socioeconomic status. **Journal of International Society of Preventive and Community Dentistry**, v. 9, n. 5, p. 513, 2019.

VOLLÚ, A. L.; BRAGANÇA, J.; RODRIGUES, G. F.; BARJA-FIDALGO, F.; FONSECA-GONÇALVES, A. Fatores comportamentais e socioeconômicos são fortes preditores de cárie dentária em pré-escolares: um estudo transversal. **Revista Científica do CRO-RJ (Rio de Janeiro Dental Journal)**, v. 7, n. 1, p. 40-48, 2022.

WALKER, A.W.; INCE, J.; DUNCAN, S.H.; WEBSTER, L.M.; HOLTROP, G.; ZE, X.; BROWN, D.; STARES, M.D.; SCOTT, P.; BERGERAT, A.; LOUIS, P.; MCINTOSH, F.; JOHNSTONE, A.M.; LOBLEY, G.E.; PARKHILL, J.; FLINT, H.J. Dominant and diet-responsive groups of bacteria within the human colonic microbiota. **The ISME Journal**, v.5, p.220-230, 2011.

WEERHEIJM, Karin L. Molar incisor hypomineralization (MIH): clinical presentation, aetiology and management. **Dental update**, v. 31, n. 1, p. 9-12, 2004.

WHATLING, R.; FEARNE, J. M. Molar incisor hypomineralization: a study of aetiological factors in a group of UK children. **International Journal Paediatr Dent**, v. 18, n. 3, p. 62-155, 2008.

WORLD HEALTH ORGANIZATION (WHO). **Information note about intake of sugars recommended in the WHO guideline for adults and children**. 2015. Disponível em: www.who.int.

WUOLLET, E.; LAISI, S.; SALMELA, E.; ESS, A.; ALALUUSUA S. Molar-incisor hypomineralization and the association with childhood illnesses and antibiotics in a group of Finnish children. **Acta Odontologica Scandinavica**, v. 74, n. 5, p. 416-422, 2016.

ANEXOS

ANEXO I - Certificado de autorização do comitê de ética em pesquisa da Universidade São Francisco (CEP/USF)

UNIVERSIDADE SÃO FRANCISCO-SP

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Relação entre a cárie na infância, microbiota bucal e hipomineralização de molar-incisivo no município de Bragança Paulista

Pesquisador: Thaís Manzano Parisotto

Área Temática:

Versão: 2

CAAE: 10408119.0.0000.5514

Instituição Proponente: Universidade São Francisco-SP

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 3.301.195

Apresentação do Projeto:

Resposta as pendências de relatoria anterior.

Objetivo da Pesquisa:

Resposta as pendências.

Avaliação dos Riscos e Benefícios:

Não se aplica.

Comentários e Considerações sobre a Pesquisa:

Em resposta às pendências os pesquisadores informaram que:

A autorização enviada e assinada pelo Secretário de Educação do município de Bragança Paulista, Prof.º Adilson Moreira Condesso é um modelo já utilizado em pesquisas anteriores aprovadas pelo CEP-USF (CAAE: 46107015.2.0000.5514 e 42997115.4.0000.5514) e que o Secretário de Educação é a autoridade que responde por todas escolas municipais de Bragança Paulista, portanto esta autorização engloba todas as escolas municipais urbanas, com crianças de 6 a 8 anos. De qualquer modo esta informação consta na carta enviada ao Prof.º Adilson, explicando o projeto, a qual encontra-se no mesmo arquivo da autorização. Foi também inserido o carimbo do Secretário de Educação na autorização, contendo seu nome completo e RG, conforme pedido do CEP.

A quantidade de crianças selecionadas inicialmente para a pesquisa é de 800 indivíduos, pois a

Endereço: Av. São Francisco de Assis, 218
Bairro: Cidade Universitária **CEP:** 12.916-900
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prevalência da alteração em questão (Hipomineralização de Molar e Incisivo) é de 10%, ou seja, estima-se que apenas 80 indivíduos desta população apresentarão o distúrbio. O n=80 já contempla as perdas de segmento durante a realização da pesquisa.

Considerações sobre os Termos de apresentação obrigatória:

Adequados.

Conclusões ou Pendências e Lista de Inadequações:

Projeto aprovado.

Considerações Finais a critério do CEP:

APÓS DISCUSSÃO EM REUNIÃO DO DIA 02/05/2019, O COLEGIADO DELIBEROU PELA APROVAÇÃO DO PROJETO DE PESQUISAS. APÓS A CONCLUSÃO DO PROJETO É OBRIGATÓRIO O ENVIO DO RELATÓRIO FINAL PARA ENCERRAMENTO DO PROJETO.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1318404.pdf	17/04/2019 19:39:07		Aceito
Outros	versao2_cartaexplicativa_e_autorizacao.pdf	17/04/2019 19:31:22	Karina Ferreira Rizzardi	Aceito
Outros	cartaresposta.docx	17/04/2019 19:30:29	Karina Ferreira Rizzardi	Aceito
Projeto Detalhado / Brochura Investigador	27_03_19_doutorado_para_comite_etica.doc	27/03/2019 10:54:11	Karina Ferreira Rizzardi	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_carie_hmi.doc	27/03/2019 10:41:22	Karina Ferreira Rizzardi	Aceito
Folha de Rosto	folha_de_rosto_assinada2019.pdf	26/03/2019 15:36:30	Karina Ferreira Rizzardi	Aceito
Outros	explicacao_seceducacao.jpg	24/03/2019 16:52:30	Karina Ferreira Rizzardi	Aceito
Outros	autorizacao.jpg	24/03/2019 16:52:03	Karina Ferreira Rizzardi	Aceito

Situação do Parecer:

Aprovado

UNIVERSIDADE SÃO FRANCISCO-SP

Continuação do Parecer: 3.301.195

Necessita Apreciação da CONEP:
Não

BRAGANCA PAULISTA, 03 de Maio de 2019

Assinado por:
Mário Angelo Claudino
(Coordenador(a))

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Karina Rizzardi <karinarizzardi@gmail.com>

Fwd: [TP] TP-23-48 submission acknowledgement

Thaís Manzano Parisotto <thais.parisotto@usf.edu.br> 29 de janeiro de 2023 às 23:11
Para: Karina Rizzardi <karina_f_r@hotmail.com>

--------- Mensagem encaminhada ---------
De: **Yu-Jia Yang** <editor@thetp.org>
Data: dom., 29 de jan. de 2023 às 21:42
Assunto: [TP] TP-23-48 submission acknowledgement
Para: Thaís Parisotto <thais.parisotto@usf.edu.br>

Dear Dr. Parisotto,

It is our pleasure to receive your manuscript "RELATIONSHIP BETWEEN CARIOGENIC BACTERIA AND MOLAR INCISOR HYPOMINERALIZATION IN THE CHILDHOOD" to Translational Pediatrics.

Your manuscript ID is: TP-23-48

With the online journal management system that we are using, you will be able to track its progress through the editorial process by logging in to the journal web site:

Manuscript URL: https://tp.amegroups.com/author/submission/108840

Username: 20230130qN66U

Thank you for contributing your article to Translational Pediatrics. If you have any questions, please contact us.

Editorial Office

_____
Translational Pediatrics
Email: editor@thetp.org
URL: https://tp.amegroups.com
--
Profa. Dra. Thaís Parisotto
Programa de Pós-Graduação em Ciências da Saúde
Universidade São Francisco - Bragança Paulista-SP.
(11)2454-8474

ANEXO II – Comprovante de submissão do artigo *"Relationship between cariogenic bacteria and molar incisor hypomineralization in the childhood"* no periódico Translational pediatrics.

APÊNDICE I - Ficha clínica utilizada na avaliação do índice de HMI e cárie

HMI e CÁRIE

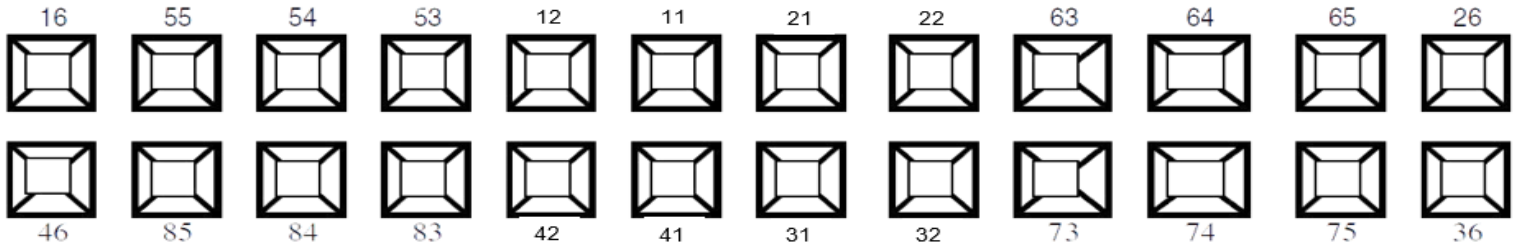
FICHA CLINICA - Nome:_____

Data nasc.:_____ Idade:_____ Sexo: (F) (M): Cor: (B) (N) (P) Presença de biofilme visível: ( ) sim ( ) não

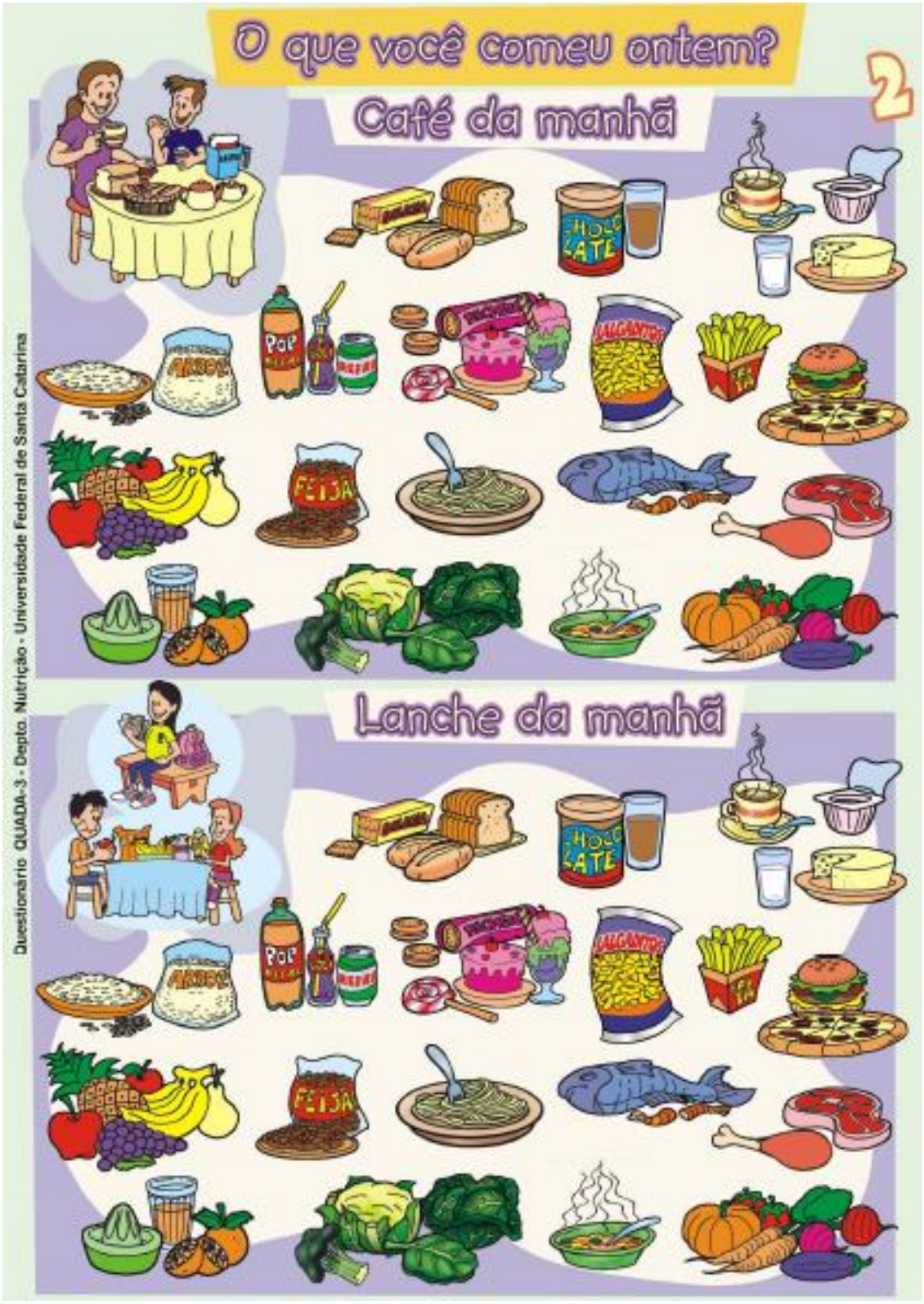
0	Esmalte livre de defeitos
1	Branco/opacidades demarcadas cremosas, sem FEP
1a	Branco/opacidades demarcadas cremosas, com FEP
2	Opacidades amarelas, marrons, demarcadas, sem FEP
2a	Opacidades amarelas, marrons, demarcadas, com FEP
3	Restauração atípica
4	Perdido por HMI
5	Parcialmente irrompido (menos de um terço da coroa) com evidência de HMI
6	Não erupcionado/parcialmente irrompido sem evidência de HMI
7	Opacidades difusas (não HMI)
8	Hipoplasia (não HMI)
9	Lesão combinada (opacidade difusa/hipoplasia com HMI)
10	Opacidades demarcadas apenas em incisivos

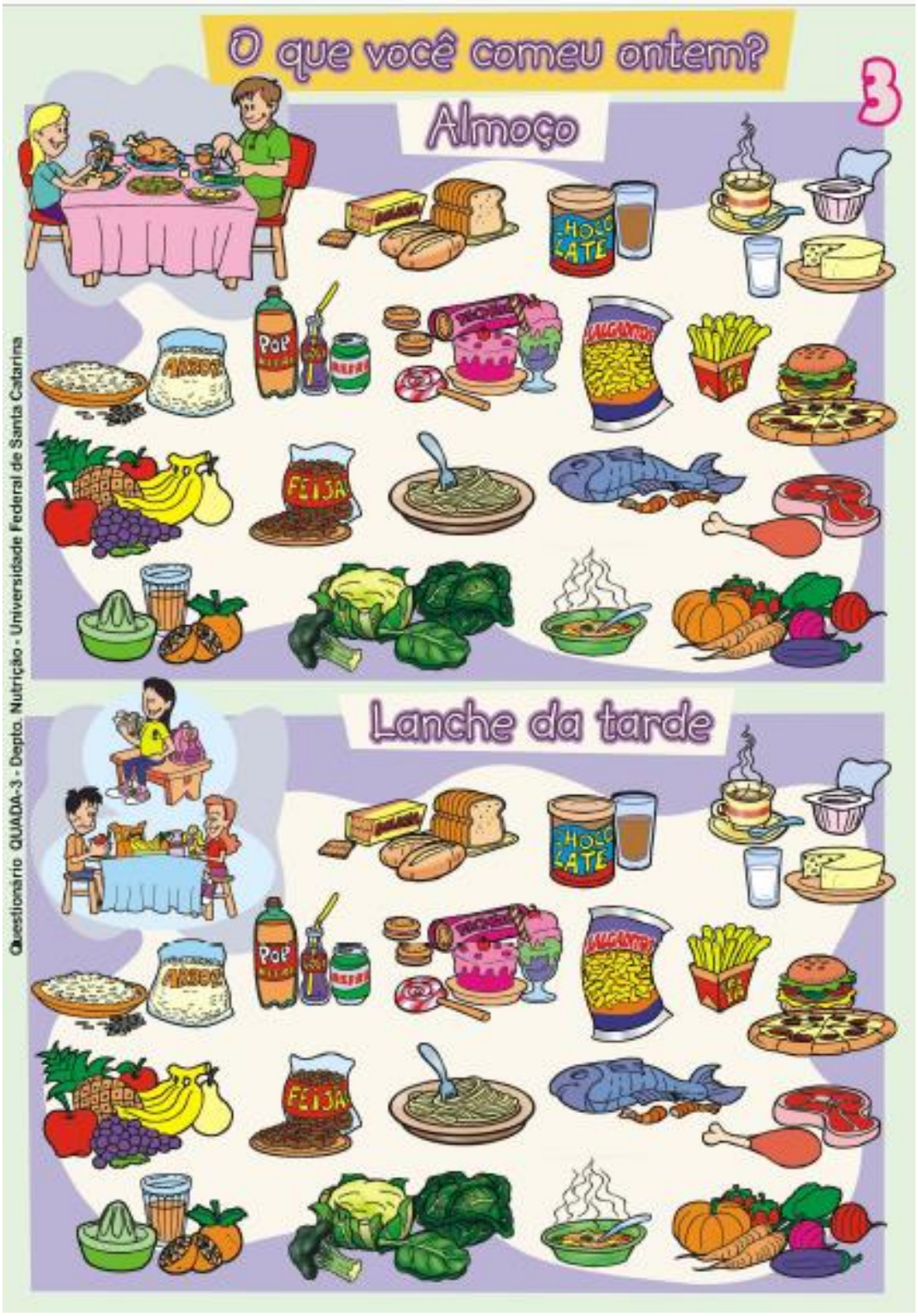
*FEP – Fratura de esmalte pós-erupção

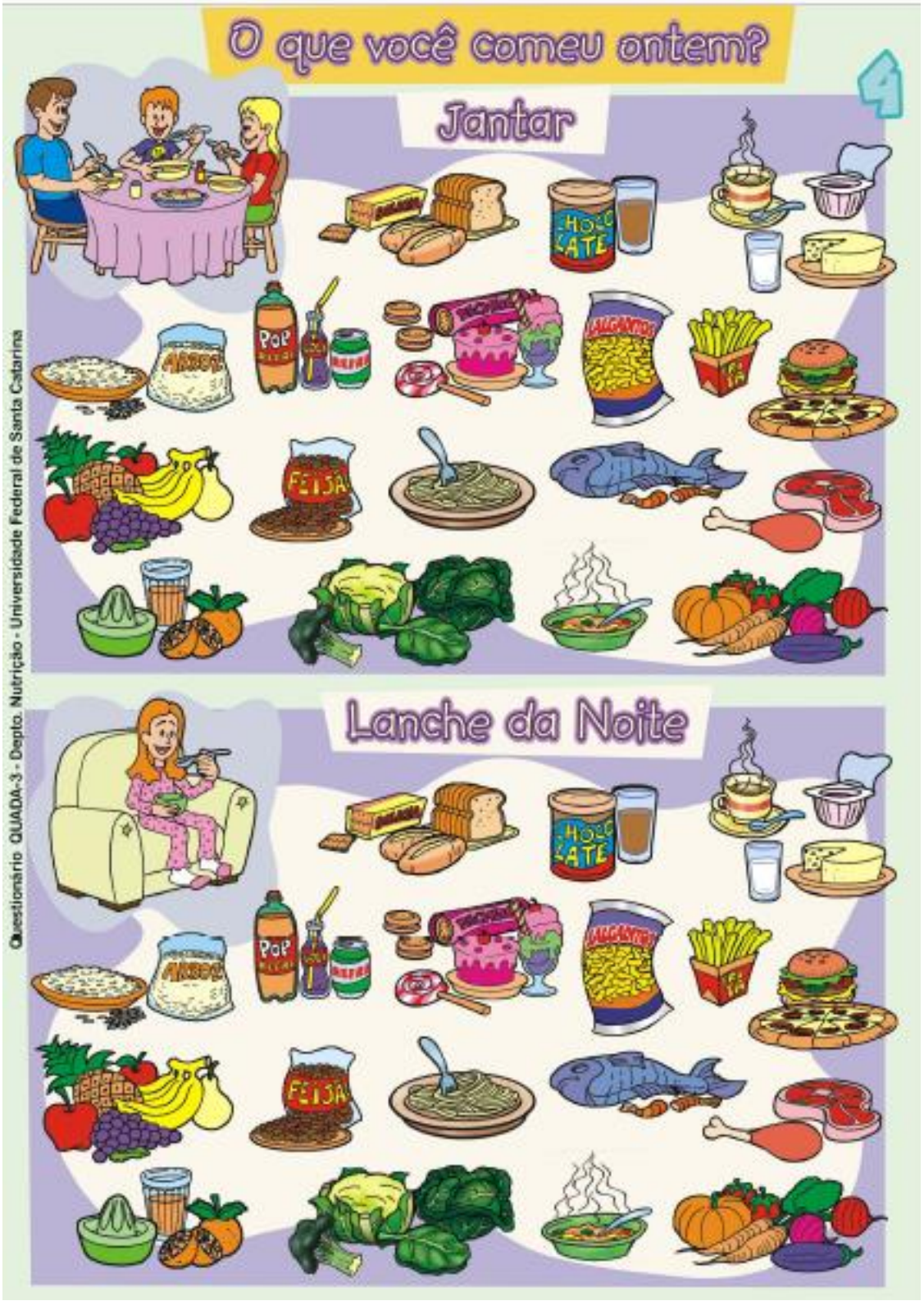
A: hígido
W: mancha branca ativa
WP: mancha branca paralisada
B: cavitado com lesão crônica
BW: cavitado com lesão ativa
C: restaurado com cavidade crônica de cárie
CW: restaurado com cavidade ativa de cárie
D: restaurado sem lesão de cárie
DW: restaurado com mancha branca
E: perdido devido à cárie

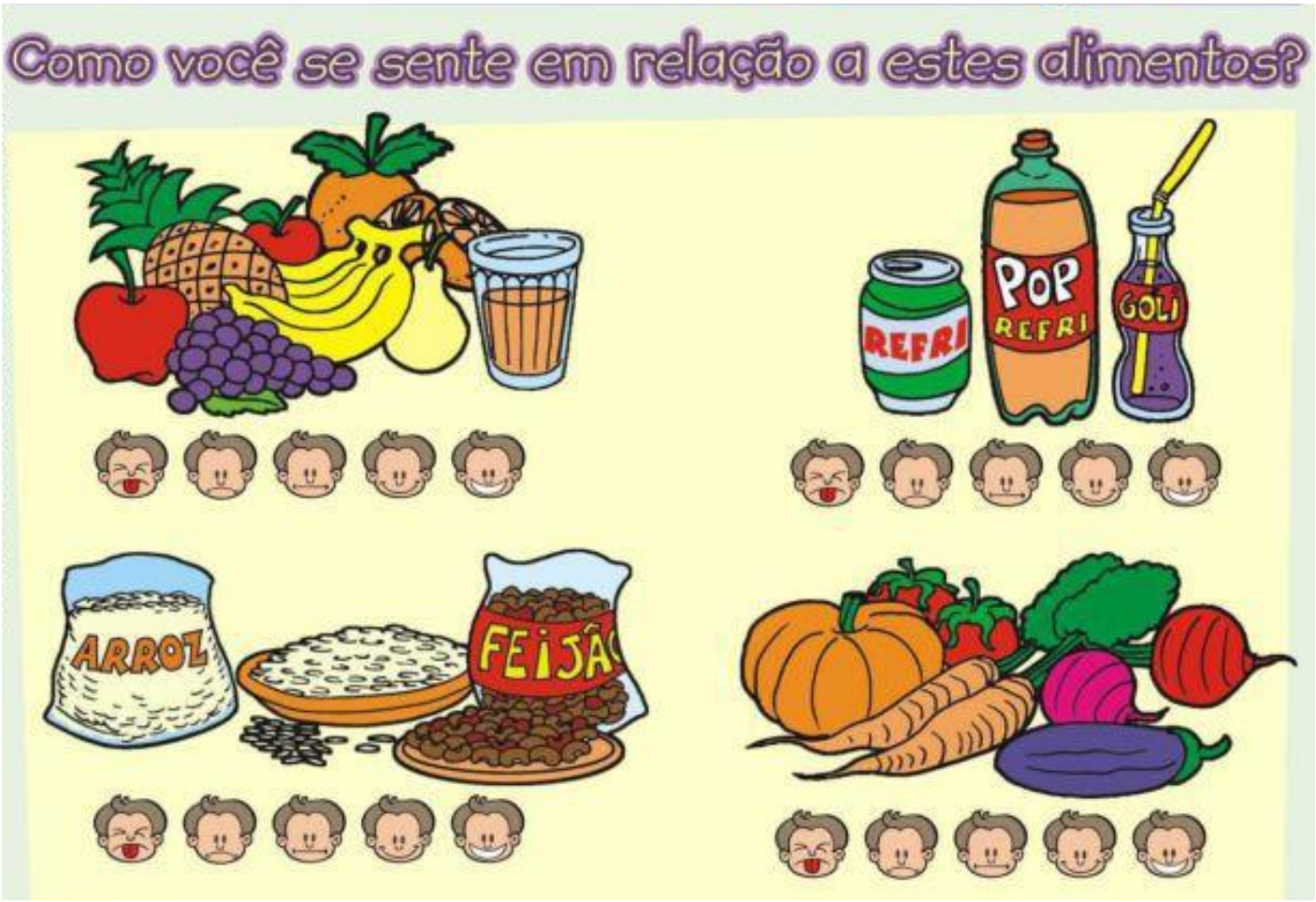


APÊNDICE II - Questionário Alimentar do Dia Anterior (QUADA-3)




O que você comeu ontem?
3
Almoço
Lanche da tarde
Questionário QUADA-3 - Depto. Nutrição - Universidade Federal de Santa Catarina


O que você comeu ontem?
4
Jantar
Lanche da Noite
Questionário QUADA-3 - Depto. Nutrição - Universidade Federal de Santa Catarina


Como você se sente em relação a estes alimentos?
REFRI
POP
REFRI
GOLI
ARROZ
FEIJÃ

APÊNDICE II - Questionário de avaliação das condições peri e pós-natais e histórico de asma/problemas respiratórios.

Problemas no último trimestre da gestação

Febre/infecção ( ) SIM ( ) NÃO

Infecção urinária ( ) SIM ( ) NÃO

Uso de antibiótico ( ) SIM ( ) NÃO

Condições perinatais – do bebê

Hipóxia (falta de oxigênio) ( ) SIM ( ) NÃO

Tipo de parto: ( ) Cesáreo ( ) Normal

Dificuldade respiratória do bebê ao nascimento ( ) SIM ( ) NÃO

Necessidade de incubadora ( ) SIM ( ) NÃO

Nascimento: ( ) Termo ( ) Pré-termo

Peso ao nascer:
( ) ≥ 4.001g;
( ) 2.501g e até 4.000g;
( ) ≤ 2.500 g.

Condições pós-natais - até o segundo ano de vida do bebê

Pneumonia ( ) SIM ( ) NÃO

Asma ( ) SIM ( ) NÃO

Sinusite / Rinite ( ) SIM ( ) NÃO

Febre ( ) SIM ( ) NÃO

Uso de antibióticos ( ) SIM ( ) NÃO