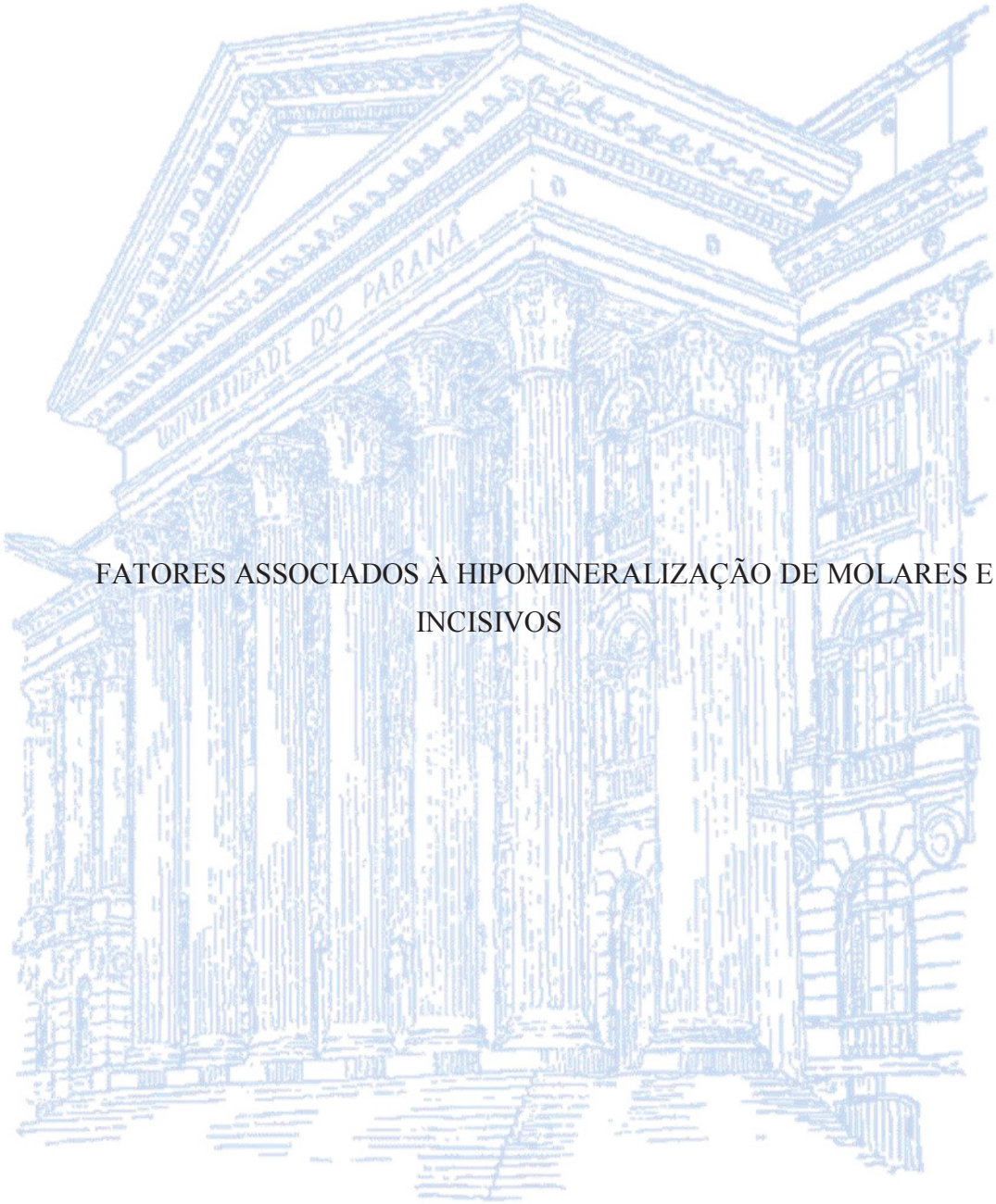
UNIVERSIDADE FEDERAL DO PARANÁ

ALUHÊ LOPES FATTURI



FATORES ASSOCIADOS À HIPOMINERALIZAÇÃO DE MOLARES E INCISIVOS

CURITIBA

2018

ALUHÊ LOPES FATTURI

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Orientadora: Profa. Dra. Juliana Feltrin de Souza Caparroz

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TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em ODONTOLOGIA da Universidade Federal do Paraná foram convocados para realizar a arguição da dissertação de Mestrado de **ALUHE LOPES FATTURI** intitulada: **FATORES ASSOCIADOS A HIPOMINERALIZAÇÃO DE MOLARES E INCISIVOS**, após terem inquirido a aluna e realizado a avaliação do trabalho, são de parecer pela sua Aprovação no rito de defesa.

A outorga do título de mestre está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

Curitiba, 18 de Julho de 2018.

JULIANA FELTRIN DE SOUZA
Presidente da Banca Examinadora (UFPR)

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Avaliador Externo (PUC/PR)

LUCIANA REICHERT ASSUNÇÃO ZANON
Avaliador Interno (UFPR)

DEDICATÓRIA

Dedico esse trabalho a minha mãe, em quem eu sempre me inspirei, ao meu pai por me dar a dádiva da vida, a minha irmã por sua cumplicidade e companheirismo e ao meu Amor pela parceira e apoio incondicional.

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Quaisquer que sejam as condições que eu tenha que enfrentar, sei que elas representam o próximo degrau na minha evolução. Aceitarei de bom grado todos os desafios, porque sei que dentro de mim estão a inteligência para compreender, o amor para aceitar e o poder para superar

Paramahansa Yogananda

RESUMO

Os defeitos de desenvolvimento de esmalte (DDE) são resultados de alterações na mineralização dentária e nas matrizes do tecido duro do esmalte durante a odontogênese (Seow, 1997). Clinicamente esses defeitos são classificados em: opacidade demarcada, opacidade difusa e hipoplasia, podendo haver variação na coloração, extensão e gravidade do DDE, sendo que, um mesmo dente pode apresentar mais de um tipo de defeito (FDI, 1992). A hipoplasia é um defeito quantitativo do esmalte, enquanto que as opacidades demarcadas, são perdas qualitativas do esmalte dentário, caracterizadas por manchas bem definidas, superfície lisa e espessura normal de esmalte (Weerheijm, Jalevik, & Alaluusua, 2001). A hipomineralização de molares e incisivos (HMI) é um defeito do esmalte específico, que se caracteriza clinicamente por opacidades demarcada de coloração variada, nos primeiros molares permanentes e frequentemente em incisivos permanentes. Quando essa hipomineralização está presente em segundos molares decíduos, esse defeito é denominado hipomineralização de segundos molares decíduos (HSMD). A etiologia desses defeitos ainda não está clara na literatura, sendo assim os objetivos desse estudo foram: (1) avaliar sistematicamente os fatores sistêmicos como exposições pré, peri e pós-natal associadas à HMI; (2) avaliar por um estudo transversal de base populacional os fatores sistêmicos como exposições pré, peri e pós natal associadas à HSMD; (3) avaliar em uma amostra de base populacional relação entre HMI e HSMD com polimorfismos genéticos no gene VDR (receptor de vitamina D). Para realizar a revisão sistemática buscas em bases de dados como PubMed, Scopus, Web of Science, LILACS, BBO, Cochrane Library e Gray, resumos da IADR, registros de ensaios inéditos, dissertações e teses de estudos observacionais que avaliaram fatores sistêmicos associados a HMI foram coletados. O risco de viés de cada estudo foi analisado, por 3 revisoras, de acordo com a escala Newcastle Ottawa. A meta-análise foi realizada considerando os dados de associação das exposição nos períodos pré-natal, perinatal e pós-natal com HMI, utilizando o Software CMA (versão 3 Biostat, Englewood, USA) (α=0,05). Um total de 4474 artigos foram identificados. Vinte e nove estudos permaneceram na síntese qualitativa. Os estudos apresentaram risco baixo e moderado de viés. Vinte e sete estudos foram incluídos para meta-análise. De acordo com a meta-análise, doença materna durante a gravidez (OR = 1,40; IC 95% 1,18-1,65; p <0,001) e estresse psicológico (OR = 2,65; IC 95%1,52-4,63; p =0,001) foram significativamente associados com maior chance de HMI. Durante o período perinatal, cesariana (OR = 1,32; IC 95% 1,11-1,57; p= 0,001) e complicações no parto (OR = 2,06; IC 95% 1,47 - 2,88, p <0,001) foram associados significantemente à HMI. No período pós-natal, as doenças respiratórias (OR 1,98; IC 95%1,45-2,70; p <0,001), febre (OR 1,50; IC 95% 2,22-1,84, p <0,001) foram associadas à maior chance de HMI. No segundo estudo observacional do tipo transversal 731 escolares de 7 a 8 anos foram selecionados aleatoriamente em Curitiba-PR. As exposições sistêmicas foram coletadas por meio de questionário estruturado aplicado às mães. A avaliação clínica das crianças foi realizada por avaliadores calibrados (Kappa> 0,80), utilizando o critério da EAPD e o índice DDE modificado. A análise de regressão múltipla de Poisson foi utilizada para avaliar as associações com abordagem hierárquica temporal das exposições sistêmicas, considerando os períodos pré-natal, perinatal e pós- natal (α=0,05). A prevalência de HSMD foi de 9,4%. No modelo múltiplo, o uso de cigarro (RP_a = 2.44; IC 1.47 – 4.06; p = 0.001) e a presença de hipertensão (RP_a = 1.73; IC 1.01 – 2.95; p = 0.044) durante a gestação aumentaram a prevalência da HSMD. Durante o período perinatal, presença de complicações no parto (RP_a 1.83; IC 1.05 – 3.19 p = 0.032) também aumentou a prevalência de HSMD independente das exposições pré-natais e demais exposições do período perinatal. A presença de otite durante os 3 primeiros anos de vida (RP_a = 1.68; IC

1.01 – 2.79 p = 0.043) aumentou a prevalência do HSMD independente das demais exposições. Para o terceiro estudo, a relação entre HMI e HSMD com polimorfismos genéticos no gene VDR (receptor de vitamina D) foi avaliada na amostra de base populacional anteriormente citada. Para tanto, os marcadores *rs22285* e *rs7398* do gene VDR foram genotipados a partir de células da mucosa bucal por meio de PCR em tempo real. Para a análise de associação os genótipos foram categorizados em modelo aditivo, alelo dominante e alelo recessivo, os quais foram analisados quanto à presença de HMI, HMI em incisivos, HSMD por meio da análise de regressão de Poisson (α=0,05). Não se observou associação entre HMI e HSMD com os polimorfismos nos marcadores *rs22285* (p>0,05) e *rs7398* (p>0,05). Porém quando se considera a presença de HMI em incisivos, crianças com genótipo GT no marcador *rs7398* apresentaram maior prevalência de HMI nestes dentes (RP=2,40; IC 95% 1,08 – 5,31; p=0,03). No modelo recessivo, a presença do alelo G (GT/GG) esteve associada à maior prevalência de HMI em incisivos (RP=2,34; IC 95% 1,08 – 5,07; p=0,03). De acordo com os estudos, pode-se concluir: que HMI apresentou associação com doenças maternas, estresse psicológico durante a gestação, parto do tipo cesárea, complicações no parto e doenças respiratórias e febre durante a infância por meio de revisão sistemática e meta-análise. Por meio de estudo transversal de base populacional, observou-se que a HSMD esteve associada às exposições pré-natais como uso de cigarro e hipertensão, bem como ao tipo de parto e complicações no parto e a presença da otite durante a infância. Ainda, observou-se que polimorfismos genéticos no gene VDR não esteve associado à presença da HMI bem como a presença da HSMD, porém observou-se associação entre a presença de HMI em incisivos com polimorfismos no marcador *rs7398*. Sugere-se que a hipomineralização demarcada como HMI e HSMD apresentam origem multifatorial, associada à exposições sistêmicas bem como genética. Estudos prospectivos são necessários para verificar a associação dos fatores sistêmicos e genéticos com a HMI e HSMD.

Palavras-chaves: Hipoplasia do esmalte dentário, Molar, Etiologia, polimorfismo genético.

ABSTRACT

Enamel development defects (DDE) are results of alterations in tooth mineralization and enamel hard tissue matrices during odontogênese (Seow, 1997). Clinically these defects are classified as: marked opacity, diffuse opacity and hypoplasia, and there may be variation in DDE staining, extent and severity, with the same tooth presenting more than one type of defect (FDI, 1992). Hypoplasia is a quantitative defect of the enamel, whereas the demarcated opacities are qualitative losses of the dental enamel, characterized by well-defined spots, smooth surface and normal enamel thickness (Weerheijm et al., 2001). The molars and incisors hypomineralization (MIH) is a specific defect of the enamel, which is characterized clinically by demarcated opacities of varied staining, in the first permanent molars and often in permanent incisors. When this hypomineralization is present in deciduous second molars, this defect is called hypomineralization of primary second molars (HSPM). The etiology of these defects is still unclear in the literature, so the objectives of this study were: (1) to systematically assess systemic factors such as pre, peri and postnatal exposures associated with MIH; (2) to assess, by a population-based cross-sectional study, systemic factors such as pre, peri and postnatal exposures associated with HSPM; (3) to evaluate in a population-based sample the relationship between MIH and HSPM with genetic polymorphisms in the VDR (vitamin D receptor) gene. To perform a systematic review, we searched databases such as PubMed, Scopus, Web of Science, LILACS, BBO, Cochrane Library and Gray, IADR abstracts, unpublished trial records, theses and theses from observational studies that evaluated systemic factors associated with MIH were collected. The risk of bias in each study was analyzed by three reviewers according to the Newcastle Ottawa scale. The meta-analysis was performed considering the association between exposures in prenatal, perinatal and postnatal periods with MIH using the CMA Software (version 3 Biostat, Englewood, USA) ($\alpha = 0.05$). A total of 4474 articles were identified. Twenty-nine studies remained in the qualitative synthesis. The studies presented low and moderate risk of bias. Twenty-seven studies were included for meta-analysis. According to the meta-analysis, maternal disease during pregnancy (OR = 1.40, 95% CI 1.18-1.65, p <0.001) and psychological stress (OR = 2.65, 95% CI, 52-4,63, p = 0.001) were significantly associated with a higher chance of MIH. During the perinatal period, cesarean (OR = 1.32, 95% CI 1.11-1.57, p = 0.001) and complications at delivery (OR = 2.06, 95% CI 1.47-2.88, p <0.001) were significantly associated with MIH. In the postnatal period, respiratory diseases (OR 1.98, 95% CI 1.45-2.70, p <0.001), fever (OR 1.50, 95% CI 2.22-1.84, p <0.001) were associated with a higher chance of MIH. In the second cross-sectional observational study, 731 students aged 7 to 8 years were randomly selected in Curitiba-PR. The systemic exposures were collected through a structured questionnaire applied to the mothers. Clinical evaluation of the children was performed by calibrated evaluators (Kappa> 0.80), using the EAPD criteria and the modified DDE index. Poisson multiple regression analysis was used to evaluate the associations with a temporal hierarchical approach of systemic exposures, considering the prenatal, perinatal and postnatal periods ($\alpha = 0.05$). The prevalence of HSPM was 9.4%. In the multiple model, use of cigarette (RPa = 2.44, CI 1.47 - 4.06, p = 0.001) and presence of hypertension (RPa = 1.73, CI 1.01 - 2.95, p = 0.044) during gestation increased the prevalence of HSPM. During the perinatal period, the presence of complications at delivery (RP 1.83, CI 1.05 - 3.19 p = 0.032) also increased the prevalence of HSPM regardless of prenatal exposures and other exposures in the perinatal period. The presence of otitis during the first 3 years of life (RP = 1.68, CI 1.01 - 2.79 p = 0.043) increased the prevalence of HSPM independently of the other exposures. For the third study, the relationship between MIH and HSPM with genetic polymorphisms in the VDR gene (vitamin D receptor) was evaluated in the above population-

based sample. To that end, rs22285 and rs7398 markers of the VDR gene were genotyped from oral mucosa cells in real-time PCR. For analysis of association, the genotypes were categorized into additive model, dominant allele and recessive allele, which were analyzed for the presence of MIH, MIH in incisors, HSPM by Poisson regression analysis ($\alpha = 0.05$). There was no association between MIH and HSPM with polymorphisms at the markers rs22285 ($p > 0.05$) and rs7398 ($p > 0.05$). However, when considering the presence of MIH in incisors, children with GT genotype in the rs7398 marker had a higher prevalence of MIH in these teeth (RP = 2.40, 95% CI 1.08 - 5.31, p = 0.03). In the recessive model, the presence of the G (GT / GG) allele was associated with a higher prevalence of MIH in incisors (RP = 2.34, 95% CI 1.08 - 5.07, p = 0.03). In this study it can be concluded through a meta-analysis that MIH showed association with maternal diseases and psychological stress during pregnancy, cesarean delivery, complications in childbirth and respiratory diseases and fever during childhood. Through a cross-sectional population-based study, it was observed that HSPM was associated with pre-natal exposures such as use of cigarette and hypertension, as well as the type of delivery and complications at delivery and the presence of otitis during childhood. Furthermore, it was observed that genetic polymorphisms in the VDR gene were not associated with the presence of MIH as well as the presence of HSPM, but an association between the presence of MIH in incisors with polymorphisms in the rs7398 marker was observed. It is suggested that hypomineralization demarcated as MIH and HSPM present multifactorial origin with systemic as well as genetic. Observational studies in other populations are required to verify the association of systemic and genetic factors with MIH and HSPM.

Key words: Dental enamel hypoplasia, Molar, Etiology, genetic polymorphism.

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BBO – Biblioteca Brasileira de Odontologia

BMW: Brazilian minimum wage

COEP – Comissão de ética em Pesquisa

DDE: Defeito de desenvolvimento de esmalte

DNA - Ácido desoxirribonucleico

EAPD : Academia Europeia de Odontologia Pediátrica

HMI: Hipomineralização de molares e incisivos

HSMD: Hipomineralização de Segundos Molares decíduos

MIH: Molar incisor hypomineralization

HSPM: Hypomineralization of Second primary molar

LILACS – Literatura Latino Americana e do Caribe em ciências da Saúde

PCR – Polymerase chain reaction

PMH: Primary molar hypomineralization

PR: Paraná

PRISMA – Preferred Reporting Items for Systematic Reviews and Meta- analyses

PROSPERO – International Prospective Register of Systematic Reviews

TCLE: Termo de Consentimento Livre e Esclarecido

UFPR – Universidade Federal do Paraná

UEPG – Universidade Estadual de Ponta Grossa

VDR – Vitamin D Receptor

VIU: Vida intrauterina

SUMÁRIO

1.INTRODUÇÃO

O processo de formação do esmalte dentário, denominado amelogênese, é um processo complexo de interação ectomesenquimal, o qual é regulado por diversos genes e fatores de crescimento (Thesleff, 2000). A amelogênese é dividida em dois estágios principais: secreção, mineralização ou maturação. No estágio secretor, os ameloblastos secretam as proteínas da matriz do esmalte, como a enamelina, ameloblastina, amelogenina, que apresentam deposição progressiva estruturadas no formato de prismas, principalmente de hidroxiapatita, formando uma matriz proteica parcialmente mineralizada (Cate, 1998). O processo de amelogênese inicia-se nas cúspides ou bordas incisais e segue em direção à região cervical (Lacruz, Smith, Kurtz, Hubbard, & Paine, 2013; Smith & Nanci, 1995), assim em um dente há diferentes estágios de desenvolvimento, sendo que na região incisal ou oclusal o processo estará mais avançado. Durante a secreção, os cristais de esmalte crescem em comprimento, assim o esmalte se desenvolve em espessura.

No estágio de mineralização ou maturação, após toda a espessura da matriz orgânica do esmalte ter sido formada, os ameloblastos sofrem significativas modificações estruturais. Assim, os ameloblastos secretores sofrem reestruturação citoplasmática, em um curto estágio de transição, a fim de garantir a degradação da matriz orgânica do esmalte. O depósito de minerais, consolida a mineralização do esmalte, que se desenvolve a medida que os cristais crescem em largura e espessura, resultando em um tecido mineralizado com 95% de seu conteúdo composto por material inorgânico (Cate, 1998; Lacruz, Smith, Kurtz, Hubbard, & Paine, 2013).

O esmalte dentário após formado não sofre remodelação, uma vez que os ameloblastos após a calcificação do esmalte sofrem diferenciação e se transformam em células epiteliais, impossibilitando a continuação da aposição mineral no dente já calcificado, por isso o esmalte dentário é considerado um importante marcador biológico (Seow, 1997). Portanto, apesar do processo da amelogênese ser controlado biologicamente, pode ser influenciado por exposições intrínsecas e

extrínsecas, as quais podem interferir nos diferentes estágios da formação do esmalte dentário, ocasionando alterações irreversíveis tanto na dentição decídua quanto na permanente (Seow, 1997).

Embora este processo seja biologicamente controlado, a amelogênese é sensível aos distúrbios ambientais, sistêmicos e genéticos, os quais afetam os ameloblastos e/ou o ambiente extracelular, podendo gerar defeitos permanentes na estrutura dentária (Alaluusua, 2010; Sonmez, Yildirim, & Bezgin, 2013). Essas alterações na formação do esmalte são denominadas defeitos de desenvolvimento do esmalte (DDE), que são alterações na aparência normal do esmalte como resultado de distúrbios ao órgão do esmalte (FDI, 1992).

Dependendo do estágio da formação do esmalte em que a alteração ocorre, as diferentes variações clinicas de DDE se manifestam. Insultos ocorridos durante o estágio da secreção da matriz do esmalte podem ocasionar alteração na espessura do esmalte, conhecida como hipoplasia do esmalte, e que, clinicamente caracteriza-se pela redução na espessura do esmalte em formato de lesões hipoplásicas como fossetas, sulcos, linhas ou até grandes regiões de ausência do esmalte (Seow, 1997; Suckling, 1989). Por sua vez, insultos ocorridos durante o estágio de mineralização ou maturação do esmalte podem ocasionar alteração na translucidez do mesmo (hipomineralização ou hipomaturação), que clinicamente se apresentam como opacidades (Seow, 1997; Suckling, 1989).

As hipomineralizações podem ser demarcadas ou difusas (Seow, 1997; Suckling, 1989). As hipomineralizações ou opacidades podem ser demarcadas, isto é, com limites bem definidos entre o esmalte normal e o esmalte hipomineralizado com a coloração variando do branco, amarelo ao marrom (FDI, 1992; Seow, 1997; Suckling, 1989), ou podem ser difusas, as quais apresentam coloração branca, sem um limite demarcado entre o esmalte normal e o esmalte afetado, que podem se apresentar em linhas, isoladas ou confluentes. As opacidades difusas têm como fator

etiológico a ingestão excessiva de fluoretos, denominada fluorose dentária (Seow, 1997; Suckling, 1989).

Opacidades demarcadas em primeiros molares permanentes, e frequentemente, em incisivos foram definidas em 2001 pela Academia Européia de Odontopediatria como hipomineralização de molares e incisivos (HMI), um tipo especifico de DDE, que se apresenta clinicamente como opacidades demarcadas envolvendo de um a quatro primeiros molares permanentes, também pode afetar os incisivos permanentes. A HMI se apresenta como um defeito qualitativo caracterizado pela alteração da translucidez normal do esmalte, conservando a normalidade da espessura e textura da superfície dentária, com distribuição assimétrica entre os dentes afetados, apresentando graus variados de gravidade entre eles (Suckling, 1989; Weerheijm et al., 2001). Nos casos mais graves pode se verificar perda estrutural pós-eruptiva do esmalte, restaurações atípicas ou em estágios mais avançados exodontia pela HMI (Fagrell, Dietz, Jalevik, & Noren, 2010; Weerheijm et al., 2001).

A HMI não é um condição nova (Weerheijm et al., 2001) visto que a análise das arcadas dentárias de esqueletos retirados do cemitério medieval de Broadgate, em Londres, revelou a presença de defeitos compatíveis com HMI (Lygidakis et al., 2008). Em 1981 tentou-se explicar a etiologia da chamada então "hipoplasia de etiologia não genética ou local" (Lygidakis et al., 2008). Esses molares eram frequentemente chamados de molares de queijo pois as lesões se assemelham clinicamente com a cor e a consistência do queijo. Outras descrições foram: hipomineralização do esmalte idiopático nos primeiros molares permanentes, opacidades do esmalte idiopático nos primeiros molares permanentes, manchas não endêmicas do esmalte nos primeiros molares permanentes, hipomineralização do esmalte não fluorótico (Kellerhoff & Lussi, 2004).

Defeito semelhante à HMI tem se observado na dentição decídua

e é denominado hipomineralização de segundos molares decíduos (HSMD) Um estudo observou que crianças com HSMD apresentam uma chance de até 6 vezes maior de terem HMI (Mittal & Sharma, 2015), em um estudo coorte com 414 crianças, observou-se que a presença de HSMD pode ser considerada um preditivo para HMI. Desta forma, a aplicabilidade clínica do diagnóstico da presença de HSMD baseia-se na alta probabilidade dessas crianças apresentarem HMI, e consequentemente, serem incluídas no grupo de alto risco a cárie dentária, necessitando de monitoramento precoce, o que permite a adoção de medidas preventivas para a preservação dos dentes afetados pela HMI (Negre-Barber, Montiel-Company, Boronat-Catala, Catala-Pizarro, & Almerich-Silla, 2016).

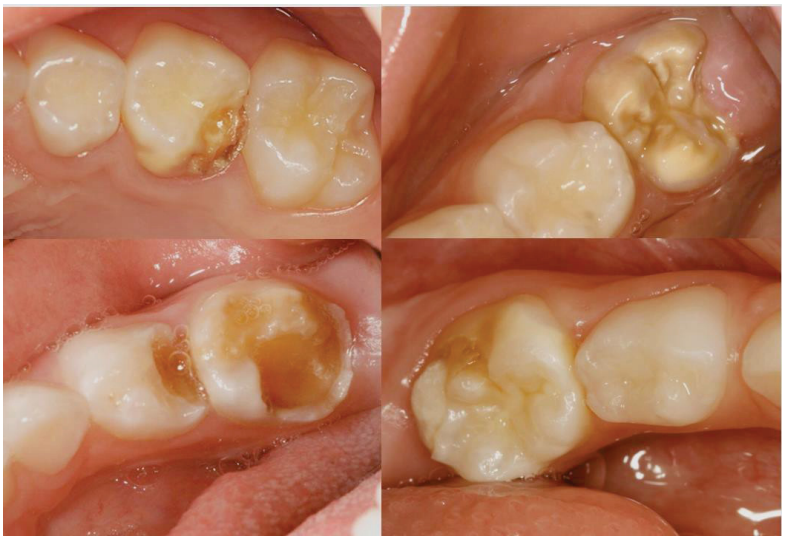

Figura 1: Características clínicas da HSMD e HMI

O desenvolvimento do segundo molar decíduo inicia-se no 4º mês de vida intrauterina, e termina por volta do 12º mês de vida da criança. Já o primeiro molar permanente inicia o processo de mineralização por volta do 5º mês de vida intrauterina e termina no terceiro ano de vida. (Whatling & Fearne, 2008). Nota-se uma coincidência cronológica de

desenvolvimento desses grupos de dentes, e portanto exposições ambientais ou sistêmicas ocorridas entre o 4º e o 5º mês de vida intrauterina até o primeiro ano de vida da criança podem resultar em hipomineralização nos segundos molares decíduos assim como no primeiro molar permanente (Temilola, Folayan, & Oyedele, 2015). Investigações sobre fatores associados devem focar nas exposições ambientais e/ou eventos genéticos ocorridos até os 3 anos de idade (Whatling & Fearne, 2008).

A etiologia da HMI e da HSMD ainda não está completamente elucidada (da Silva Figueiredo Se et al., 2017). Durante o desenvolvimento dentário uma série de fatores pode interagir de forma acumulada ou combinada afetando os ameloblastos e perturbando a formação da matriz ou maturação do esmalte. Diversos fatores têm sido sugeridos como associados ao DDE em dentes decíduos como problemas nos períodos pré, peri e pós natais, podendo ser problemas sistêmicos ou locais e condições genéticas. Exposições relacionadas à mãe durante a gestação como: idade da mãe no nascimento, influências sociais, doenças ou infecções durante a gestação, má nutrição, uso de medicamentos, consumo de álcool e tabagismo. Além disso, condições relacionadas à criança tais como prematuridade, baixo peso ao nascimento, índice Apgar, febre, infecções e outras doenças, falta de aleitamento materno ou aleitamento materno prolongado, uso de antibiótico, doenças respiratórias, doenças infecciosas, fatores sociais, demográficos e comportamentais, deficiências nutricionais nos períodos pré ou pós-natais, e alterações genéticas podem aumentar o risco aos DDE (Allazzam, Alaki, & El Meligy, 2014; Mishra & Pandey, 2016; van der Tas et al., 2018; Wagner, 2016). Estudos em animais corroboram com as observações epidemiológicas ao demonstrar que algumas alterações sistêmicas, biologicamente, também poderiam perturbar a amelogênese, como febre e uso de antibióticos (Tung, Fujita, Yamashita, & Takagi, 2006; Wuollet, Laisi, Salmela, Ess, & Alaluusua, 2014).

Em estudos de revisão sistemática, Crombie et al., Alaluusua e Silva et al, investigaram as exposições associadas à HMI, incluindo exposições durante os períodos pré-natal, perinatal e pós-natal. Os autores observaram que crianças com problemas respiratórios, complicações durante o parto, baixo peso ao nascer, falta de oxigênio ao nascer, distúrbios metabólicos e de cálcio e fosfato, asma, infecções do trato respiratório, febre alta e consumo de antibióticos podem aumentar o risco da HMI (Alaluusua, 2010; Crombie, Manton, & Kilpatrick, 2009; Silva, Scurrah, Craig, Manton, & Kilpatrick, 2016)

Sabe-se que a vitamina D está intimamente envolvida na formação dos dentes (Descroix, Kato, Lezot, & Berdal, 2010), não apenas como um regulador da homeostase mineral, mantendo uma relação estável entre os íons fosfato e cálcio, que podem influenciar a qualidade do osso, esmalte e dentina, mas também estando envolvida na resposta imune do indivíduo. A deficiência de vitamina D pode provocar as alterações descontroladas no sistema imunológico, que bloqueia a resposta imune correta à microbiota bucal (Holla et al., 2017; Kong et al., 2017). A principal função da vitamina D é manter as concentrações plasmáticas de cálcio em um nível constante, o que é importante para o desenvolvimento ósseo saudável, bem como o desenvolvimento saudável dos dentes. A vitamina D estimula a mineralização do esmalte dental por ligação a receptores que são expressos em ambos os tecidos ósseo e dentário (van der Tas et al., 2018). Como os ameloblastos e odontoblastos são células-alvo da vitamina D, é plausível afirmar que a deficiência de vitamina D está ligada a distúrbios do desenvolvimento do esmalte (Kuhnisch et al., 2015).

Estudos em animais mostraram que a interrupção da via da vitamina D leva a níveis inadequados de cálcio e fósforo no plasma circulante, resultando na diminuição da mineralização óssea e um impacto negativo na mineralização dos dentes (Zhang, Beck, Rahemtulla, & Thomas, 2009)

O receptor da vitamina D (VDR) é considerado como um mediador para o efeito da mineralização relacionada à vitamina D (Yu, Jiang, Sun, Kong, & Chen, 2017). O gene VDR está localizado no cromossomo 12q13.11 e contém várias regiões polimórficas (Holla et al., 2017), modulando a função biológica dos principais metabólitos da vitamina D, portanto, desempenha um papel importante na formação dos dentes, particularmente na calcificação do esmalte dentário (Kong et al., 2017). O mau funcionamento da via da vitamina D no organismo pode ser resultado da deficiência de vitamina D ou da mutação do gene VDR (Descroix et al., 2010). O que pode alterar os níveis ideais de minerais para a mineralização dentária. Sabe-se que variações no genoma humano do VDR podem causar malformações hereditárias do esmalte dentário (Yu et al., 2017).

Embora inúmeros fatores etiológicos sistêmicos e ambientais tenham sido associados aos defeitos do esmalte, estudos mais recentes sugerem que a predisposição genética também tem um papel importante na etiologia da HMI (Jeremias et al., 2013; Vieira & Kup, 2016). Estudos recentes sugerem uma predisposição genética, na qual as variações genéticas em proteínas expressas durante a amelogênese são fundamentais para a etiologia do HMI (Vieira & Kup, 2016). Diante do exposto, frente a semelhança clínica entre HSMD e HMI (Figura 1), bem como a coincidência cronológica de desenvolvimento, acredita-se que os possíveis fatores etiológicos podem ser coincidentes para hipomineralizações em ambas as dentições (Elfrink et al., 2012; Ghanim, Morgan, Marino, Bailey, & Manton, 2012). Nota-se que na literatura diversos estudos têm investigado sobre as exposições sistêmicas associadas à HMI (Alaluusua, 2010; Crombie et al., 2009; Sidaly et al., 2016; Silva et al., 2016; Souza et al., 2012; Wuollet et al., 2014), e poucos estudos sobre a etiologia da HSMD (Elfrink, Schuller, Weerheijm, & Veerkamp, 2008; Ghanim, Manton, Marino, Morgan, & Bailey, 2013; Mittal & Sharma, 2015; Negre-Barber et al., 2016; Oyedele, Folayan, & Oziegbe, 2016). A partir da premissa de que o nível inadequado de vitamina D pode induzir defeitos

no esmalte durante o desenvolvimento dentário (Uwitonze et al., 2018), esse trabalho tem por objetivo investigar as exposições sistêmicas relacionadas à HMI de acordo com a evidência disponível na literatura e investigar por meio de um estudo transversal as exposições associadas à HSMD, bem como se os polimorfismos no gene VDR estão associados à prevalência de HMI e HSMD.

2. OBJETIVO

Avaliar se fatores ambientais, locais ou sistêmicos e polimorfismos genéticos estão associados à hipomineralização demarcada nos períodos pré, peri e pós natais

2.1. OBJETIVOS ESPECÍFICOS

1. Realizar uma revisão sistemática e meta-análise para verificar as exposições sistêmicas que podem estar associadas à HMI.
2. Avaliar por meio de um estudo observacional transversal com abordagem hierárquica as exposições sistêmicas associadas à HSMD.
3. Avaliar em uma amostra de base populacional relação entre HMI e HSMD com polimorfismos genéticos no gene VDR (receptor de vitamina D).

3. ARTIGO 1

A systematic review and meta-analysis of systemic exposure associated with Molar Incisor Hypomineralization

Esse artigo foi formatado de acordo com as normas do periódico Community Dentistry and Oral Epidemiology

Abstract

Background: Although there are several studies about the systemic factors associated to Molar Incisor hypomineralization (MIH), a meta-analysis study of these data was found in the literature. The MIH etiology is still unclear. **Objective:** to evaluate systemic exposures associated with Molar Incisor Hypomineralization (MIH). **Methods:** This systematic review was performed through the survey of observational studies that evaluated the systemic exposure factors associated with MIH. The sources of articles searched were PubMed, Scopus, Web of Science, LILACS, BBO, Cochrane Library and Grey literature. The risk of bias was analyzed according to the Newcastle-Ottawa Scale for quality assessment. The meta-analysis was performed considering the exposure during the prenatal, perinatal and postnatal periods using the CMA software. **Results:** A total of 4207 articles was identified. Twenty-nine studies were eligible for inclusion and twenty-seven were included in the meta-analysis. The studies presented low and moderate risk of bias, except for one that was classified at high risk of bias. Maternal illness during pregnancy (OR 1.40; 95% CI 1.18-1.65, p< 0.0001), and psychological stress (OR = 2.65; 95% CI 1.52-4.63; p= 0.001) were observed to be significantly associated with higher prevalence of MIH. During the perinatal period, cesarean delivery (OR = 1.32, 95% CI 1.11-1.57, p=0.001) and delivery complications (OR = 2.06; 95% CI 1.47-2.88, p< 0.0001) were also associated with MIH. In the postnatal period, only respiratory diseases (OR = 1.98; 95% CI 1.45-2.70, p < 0.0001) and fever (OR = 1.504; 95% CI 1.22-1.84; p< 0.0001) were associated with higher prevalence of MIH. The evidence was graded as very low quality. **Conclusions**: Maternal illness, psychological stress, cesarean delivery, delivery complications, respiratory diseases and fever during the first years of a child's life were significantly associated with higher prevalence of MIH. However, this should be interpreted with caution due to the very low quality of the evidence, once the primary studies were observational, with serious limitations according to risk of bias, imprecision and inconsistency. Further well-designed cohort studies are still required.

Key-words: Molar Incisor Hypomineralization, Dentition, Etiology, Systematic review, Meta-analysis.

Introduction

Molar-incisor hypomineralization [MIH] was defined as a specific dental enamel defect with systemic origin that affects at least one permanent molar and often permanent incisors.[1] Clinically, it is characterized by well-defined opacities, and post-eruptive breakdown occurs in severe cases favoring the development of dental caries lesions, dental sensitivity and discomfort to the patient.[2] Currently, MIH is the most frequently enamel defect that is observed asymmetrically in the first permanent molars, and frequently in incisors.

The etiology of MIH is still unclear, studies have pointed that systemic exposures such as fever, infections, stress and respiratory problems that affect the amelogenesis contribute for permanent dental structure defects.[3, 4] Most of the recent literature on the MIH etiology are based on retrospective study designs in which the knowledge of which exposures were involved in this enamel defect was uncertain [5, 6]. Many studies report its etiology based on the use of antibiotics,[7, 8, 9] others report that the occurrence of fever during pregnancy[10, 11, 12] or fever in the first years of the child's life,[13, 14] use of cigarette during pregnancy[5, 15], maternal use of alcoholic drink,[14] stress[16] and respiratory infections[4, 7, 13] such as asthma and pneumonia also predict MIH.

Some systematic review studies[3, 17, 18], investigated the systematic exposures associated with MIH, including exposures during the prenatal, perinatal and postnatal periods. Children with respiratory problems, complications during delivery, low birth weight, lack of oxygen at birth, metabolic and calcium and phosphate disturbances, asthma, respiratory tract infections, high fever and antibiotics consumption were associated with increased presence of MIH.[11, 12] Although none of these studies reached the conclusions of which the specific factors involved with the etiology of the MIH are, none of these studies carried out a meta-analysis, which has the advantage of determining how strong the association of the predictor factor and the occurrence of MIH is.

Thus, the objective of this study was to perform a systematic review of the literature to identify exposure factors that are associated with MIH, analyzing the force of the associations through meta-analysis. Therefore, the purpose of this systematic review and meta-analysis was to answer the research question: Are there systemic conditions during the prenatal, perinatal and postnatal periods associated with MIH?

. Materials and methods

2.1 Protocol and registration

This study protocol was registered in the PROSPERO database (protocol number CRD42016035741) and the recommendations of the PRISMA statement were followed for the report of this study.[19] This study was accomplished from August 2017 to March 2018 at the Federal University of Paraná, Paraná, Brazil and State University of Ponta Grossa, Paraná, Brazil.

2.2 Information sources and search strategy

We defined a search strategy based on controlled vocabulary (MeSH terms) of the PubMed database along with free keywords. These words were combined with the Boolean operator "OR" within each concept of the search strategy. The concepts were then combined with the Boolean operator "AND" (Table 1). Other electronic databases (Scopus, Web of Science, the Latin American and Caribbean Health Sciences Literature database [LILACS], the Brazilian Library in Dentistry [BBO] and the Cochrane Library) were also used. The search strategy of PubMed was adapted for each of these databases (Table 1). We also hand-searched the reference lists of all primary studies for additional relevant publications and investigated the related article links for each primary study in the PubMed database. No restrictions on publication date or languages were involved.

Abstracts of the International Association for Dental Research (IADR) and its regional divisions (1990–2017) were used and the grey literature was explored using the database System for Information on Grey Literature in Europe (SIGLE) and Google Scholar. Dissertations and theses were searched using the ProQuest Dissertations and Theses Full Text data bases and the Capes Theses database.

2.3 Eligibility Criteria

The types of studies included were cohort, cross-sectional, and case-control studies in children that evaluated the association of prenatal, perinatal and postnatal systemic factors with MIH. We excluded 1) animal studies, 2) studies that evaluated the association of systemic factors with other enamel defects in general.

2.4 Study selection and data collection process

The articles were selected by title and abstracts according to the criteria previously described. Articles found in more than one database were considered only once. The full text of the articles was obtained when there was insufficient information in the title and abstract to decide about the inclusion of the article.

Three reviewers (A.L.F, J.F.S and L.M.W) evaluated the full-text articles and selected those that met the eligibility criteria. Relevant information about the study design, number and characteristics of the participants, method to obtain the data and results were extracted by the authors (A.L.F, J.F.S and L.M.W) (Table 2). The collection form was tested on a sample of study reports used as a pilot sample to ensure that the form was consistent with the research question.

2.5 Risk of bias

The risk of bias of the studies included was evaluated by three reviewers (A.L.F, J.F.S and L.M.W) using the Modified Newcastle - Ottawa Scale for Risk of Bias criteria.[20] Discrepancies between the examiners were solved in a consensus. The evaluation criteria presented a maximum score of 9 points. These score points were divided between the following domains: patient selection (generalization and applicability - 4 points), comparability of groups (2 points), exposure measurements in studies designed (3 points). The studies were later classified as high risk of bias (0-3 points), moderate risk of bias (4-6 points) and low risk of bias (≥7 points).[21]

2.6 Summary measures and synthesis of the results

The eligible studies usually reported a wide variety of exposures; however, a meta-analysis of the data was performed of the most frequent ones in all studies. The result of interest was the frequency of presence or absence of MIH in relation to the systemic factors for each patient, while the severity of MIH was not considered in this study. For the meta-analysis, data from the studies included that presented similar exposures in the prenatal, perinatal and postnatal was merged.

During the prenatal period, we investigated the impact of maternal medicine use (vitamins, analgesics, hypertension medication, anticonvulsants, thyroid remedies, antibiotics, and medicines to prevent premature birth), maternal illness (hypertension, anemia,

toxoplasmosis, urinary infection, diabetes, renal disease, pre-eclampsia), maternal use of cigarette, psychological stress and maternal use of alcoholic drink. In the perinatal period, the following exposures were investigated: caesarean birth, prematurity, low birth weight (born under 2.5 kg), delivery complications and breastfeeding. For delivery complications, any type of problem during birth or baby hypoxia were considered.

Finally, for the postnatal period, the following exposures were investigated: jaundice, respiratory diseases (asthma, bronchitis, rhinitis and pneumonia), infections (tonsillitis, otitis, urinary tract infection, gastroenteritis and throat infection), fever (above 39 Celsius degree), childhood illness (anemia, high levels of vitamin D, urinary infection, diarrhea, rubella and varicella) and use of antibiotics.

We used random effects meta-analysis to estimate the pooled odds ratio (OR) for the association between each systemic factor and MIH across studies. We chose random rather than fixed effects models because we expected a high level of heterogeneity across studies and standard errors estimated using random effects models are generally more conservative than those estimated with fixed effects models. The whole analysis was carried out using CMA software (version 3, Biostat Englewood, USA). We restricted the meta-analysis to studies that were classified as having a moderate or low risk of bias.

2.7 Assessment of the quality of evidence using GRADE

We graded the quality of the evidence for each outcome across studies (body of evidence) using the Grading of Recommendations: Assessment, Development and Evaluation (GRADE) (http://www.gradeworkinggroup.org/). This technique allows to determine the overall strength of evidence for each meta-analysis.[22] Using the GRADE framework, the body of evidence for observational studies is initially classified as low quality. This body of evidence can be rate up if there study presents special strengths. A factor that may rate up the quality of evidence for observational studies is the presence of a large magnitude of an effect (one or two-level upgrade), presence of a dose-response gradient (one-level upgrade) and by the effect of a plausible residual confounding (one-level upgrade). However, the decision to rate up the quality of evidence should only be applied if no serious limitations are observed in the five areas that reduce the quality of the evidence (risk of bias, imprecision, inconsistency, indirectness and effects of residual confounding).[23]

Results

3.1. Study Selection

The initial search in the databases and other searches resulted in 4207 studies. The removal of duplicates resulted in 3531 studies. After checking the title of the articles, 100 studies were selected. Sixty studies were further excluded after reading the abstract, resulting in 40 full-text articles for the eligibility evaluation (Figure 1).

Eleven studies were excluded for: 1) being literature reviews[24]; 2) evaluating defects of enamel in deciduous molars[25, 26, 27]; 3) lacking a control group[28, 29] 4); having assessed association of risk factors with amelogenesis[30]; 5) attempting to associate MIH with genetic and non-systemic factors[6, 31, 32]; 6) being a case report[33] (Figure 1).

3.2 Characteristics of included articles

The characteristics of the 29 eligible studies are presented in Table 2. Thirteen were cross-sectional studies[4, 7, 8, 10, 14, 15, 16, 34, 35, 36, 37, 38, 39], ten were case-control studies[11, 12, 13, 40, 41, 42, 43, 44, 45, 46] and six were retrospective studies.[5, 9, 47, 48, 49, 50] The mean age of all the participants included in the studies was approximately 9 years old. The number of patients included in the studies varied from 45 to 4049 children. The number of patients per group included in the primary studies with MIH ranged from 14 to 262 and non MIH from 14 to 3522.

Sixteen studies enrolled patients from schools[4, 5, 7, 8, 9, 11, 12, 14, 15, 16, 36, 37, 39, 40, 43, 47], five studies from university clinics[10, 35, 41, 42, 46], four studies enrolled patients from hospitals[13, 36, 48, 49] and two studies enrolled patients from dental clinic[44, 45] .

Data were obtained through questionnaires answered by the parents in 18 studies[4, 5, 7, 10, 11, 12, 14, 15, 16, 34, 37, 38, 39, 40, 41, 46, 47, 48], questionnaire and interview with parents in four studies[8, 13, 35, 43], questionnaire to parents and medical records in five studies[36, 42, 45, 49, 50] and medical records in two studies.[9, 44]

Most of the studies (n = 21), used diagnostic criteria by the European Academy of Pediatric Dentistry (EAPD)[4, 5, 7, 9, 10, 11, 14, 15, 16, 35, 36, 37, 39, 41, 42, 43, 45, 46, 48, 49], three studies used

the modified DDE index[8, 12, 36, 40] and four studies did not specify the diagnostic criteria used.[13, 34, 38, 44]

3.3 Assessment of the risk of bias

The risk of bias assessment in the studies is presented in Table 2. One study presented high risk of bias [38] that was excluded. Sixteen studies presented moderate risk of bias[5, 7, 8, 9, 10, 13, 16, 34, 35, 36, 41, 42, 44, 47, 48, 49], and 12 studies showed low risk of bias[4, 11, 12, 14, 15, 36, 37, 39, 40, 43, 45, 46], according to the Newcastle–Ottawa Scale quality assessment.

3.4 Meta-analysis

Two studies were not included in the meta-analysis[46, 48] because data not could be extracted. For the meta-analysis, we merged data from the studies included that presented similar exposures in the prenatal, perinatal and childhood illness. Sixteen meta-analyses were conducted with the aim to associate specific risk factors with MIH.

A total of 5 meta-analyses were conducted for exposures in the prenatal period (Figure 2): 1) maternal medicine use (Figure 2A); 2) maternal illness (Figure 2B); 3) maternal use of cigarette (Figure 2C); 4) maternal alcoholism (Figure 2D); and 5) psychological stress (Figure 2E).

Maternal medicine use (OR 1.10; 95% CI 0.78-1.54; p= 0.56), maternal use of cigarette (OR = 1.20; 95% CI 0.98-1.47; p= 0.06), and maternal alcohol (OR = 1.16; 95% CI 0.06-21.6; p= 0.91) were not associated with MIH (Figure 2). On the other hand, the presence of maternal illness (OR = 1.40; 95% CI 1.18-1.65, p< 0.0001) was associated with 40% higher odds of MIH. Psychological stress (OR = 2.65; 95% CI 1.52-4.63; p= 0.001) during pregnancy was associated with 165% higher chance of MIH (Figure 2).

Heterogeneity of data was observed in the meta-analysis of maternal medicine use (chi-square test, p = 0.029; I^2 = 59.9) and maternal alcoholism (chi-square test, p = 0.028;I^2 = 79.3).

Other five meta-analyses were conducted for the perinatal period (Figure 3). The cesarean exposure (Figure 3A); prematurity exposure (Figure 3B); low birth weight (Figure 3C); delivery complications (Figure 3D); and breastfeeding (Figure 3E). Cesarean exposure

was associated with MIH, presenting 32% higher chance of MIH (OR = 1.32, 95% CI 1.11-1.57, p= 0.001) and delivery complications was associated with MIH (OR = 2.06; 95% CI 1.47-2.88, p< 0.0001). Prematurity exposure (OR 1.22; 95% CI 0.87-1.70; p= 0.24), low birth weight (OR = 1.52; 95% CI 0.83-2.79; p= 0.17) and breastfeeding (OR = 1.15; 95% CI 0.95-1.40, p= 0.14) were not associated with increased odds of MIH.

Data heterogeneity was observed in the meta-analysis of low birth weight (chi-square test, p< 0.0001, $I^2 = 83.9$) and prematurity exposure (chi-square test, p= 0.123, $I^2 = 33.3$).

Six meta-analyses were conducted for the postnatal period (Figure 4). The occurrence of jaundice (Figure 4A), respiratory diseases (Figure 4B), infections (Figure 4C), fever (Figure 4D), childhood illnesses (Figure 4E), and antibiotics exposure (Figure 4F). Respiratory diseases (OR = 1.98; 95% CI 1.45-2.70; p< 0.0001) were associated with higher chance of MIH. The presence of fever was associated with 50% higher chance for MIH (OR = 1.50; 95% CI 1.22-1.84; p< 0.0001). Other exposure factors such as jaundice (OR = 1.23; 95% CI 0.73-2.07; p= 0.42), infections (OR = 1.47; 95% CI 0.78-2.76, p= 0.22), childhood illnesses (OR 1.42; 95% CI 0.69-2.91; p= 0.34) and antibiotics exposure (OR = 1.28; 95% CI 0.99-1.65; p= 0.05) were not associated with increased odds for MIH.

Heterogeneity was observed for the meta-analysis of the association between MIH and respiratory disease (chi-square test, p< 0.0001; $I^2 = 79.9$), MIH and infections (chi-square test, p< 0.0001; $I^2 = 88.0$), fever (chi-square test, p= 0.08; $I^2 = 0.08$), childhood illnesses (χ^2 test, p < 0.0001; $I^2 = 85.6$), and antibiotics exposure (chi-square test, p = 0.03; $I^2 = 51.3$).

3.5 Assessment of the quality of the body of evidence.

According to the GRADE approach, observational studies are considered of low level of evidence. The quality of the evidence could not be rated up as serious limitations were observed in regard to the risk of bias of the studies included. All meta-analyses herein presented were performed in studies with moderate or low risk of bias. Studies are considered of moderate quality when they carry some important bias in their results. This fact decreased in one level the quality of the evidence of all meta-analyses to very low quality. Additionally, imprecision (high confidence interval of the estimate) and inconsistency (heterogeneity) were other important limitations of some meta-analyses. Imprecision was observed in the meta-

analyses of maternal alcohol and psychological stress. Inconsistency was observed in the meta-analyses of maternal medicine use, maternal use of alcoholic drink, low birth weight, respiratory diseases, infections and childhood illnesses.

Discussion

Systematic reviews and meta-analyses may solve the problem of controversies among studies and increase the power of any research question. They also may allow researchers to evaluate critically the body of evidence and summarize it for the development of recommendations for future studies that is why systematic reviews are at the top of the pyramid of scientific evidence.[51]

The search for the MIH etiology is complex, once there is a long time interval from the enamel development and the MIH diagnosis. Nowadays, the MIH origin is defined as a complex disease, in which systemic and genetic factors act in synergism for the development of HMI.[52] However, the systemic exposures involved in the MIH etiology are among the most studied topics during the last few years and it is yet unclear.[18] Although several systematic reviews were conducted with the aim to identify exposure factors involved with MIH[17, 18, 53], this is the first systematic review that conducted meta-analyses to evaluate the association between prenatal, perinatal and postnatal factors with MIH. Additionally, in the present study, a broader search strategy was employed in different databases without language restrictions, while in the earlier systematic review, the authors restricted their searches to Pubmed and Embase.

In the prenatal period, use of medicines during pregnancy, maternal use of cigarettes and maternal alcohol were not associated with increased prevalence of MIH. The exposure to medicine during pregnancy, the literature presented information on the use of a wide variety of drugs during pregnancy.[17, 54] Thus, for this meta-analysis, we merged maternal exposure to medicines in general, from vitamins to medicines for diabetes, hypertension and antibiotics like amoxicillin, this may explain the discrepancies of this exposure among studies, and in some of them this category appears as a protection factor, it was not possible to evaluate each medication separately.

The use of cigarettes during pregnancy was not associated to MIH. It is important to state that there were many methods to measure the exposure to cigarettes, as observed in the studies. Most of the studies evaluated the presence or absence of use of cigarettes. Only Souza et al[11] determined the frequency of the use of cigarettes. In this category, maternal use of cigarette included studies of mothers who smoked 1 cigarette a day and others that smoked over 20 cigarettes a day.

Considering the alcohol consumption during pregnancy and MIH, only two studies could be included in the meta-analysis, showing controversial findings[14 12], which resulted in a high heterogeneity. The MIH diagnosis may be one of the probable factors of the controversial findings, while Pitiphat et al [14] used the criteria from EAPD 2003[1], Rodrigues et al[12] used the DDE-modified index, which was not specifically developed for MIH diagnosis.

The maternal illness prenatal factor was associated with the development of MIH. This meta-analysis showed that children whose mothers had health problems during gestation presented a 40% higher chance of having MIH than mothers that had no problems in gestation. Although heterogeneity was not observed. The role of this condition or disease on the enamel development was not clear in the literature. However, some conditions are known to alter the extracellular environment, for example, the fever, interfering on the ameloblasts activities.[55] In an animal study, Tung et al. induced a febrile state for 57h, they observed alterations on the shape of enamel prisms in most of the animals. The authors suggested that the severity of the disturbance of the enamel formation was swayed by the intensity of the stress and the host's sensitivity to the physical alteration.

Similarly, a positive association between psychological stress during gestation and MIH was observed, although this information was only based on two studies[16, 43], they were conducted in countries with constant wartime tensions, South Korea and Iraq, which may explain this exposure having a significant effect. Stress and anxiety are psychological conditions that are associated with physical changes[56], such as change in nutritional status, sleep disturbance, weight loss and other unknown conditions. These physical changes may favor the association with enamel defects.[43]

From the exposure factors related to the perinatal period, cesarean and delivery complications were associated with MIH. Considering that cesarean is the delivery of choice

when the pregnancy presents some risk, such as preeclampsia, prematurity, mother high pressure or diabetes[57], a confounding factor may exist between cesarean delivery and MIH risk. Perhaps the association of delivery complications and cesarean procedures with MIH may be related to hypoxia during birth, which was shown to be a risk factor for enamel defects.[42]

Prematurity, breastfeeding and low-birth weight were not associated with MIH. The report of prematurity varied according to the mothers' understanding in some studies, Tourino et al.[7], for instance, asked in their questionnaire only if birth had been premature. On the other hand, Souza et al[15] were more specific asking if birth occurred at or below 7 months of gestation. Prematurity for Sonmez et al[4] was when birth occurred below 37 weeks of gestation. This variety in measuring prematurity does not provide a direct and precise analysis of the impact of premature birth and MIH, explaining the data heterogeneity of this finding. Similarly, there was a lack of standardization in the low birth weight information. The majority of the reports did not present the cut-off point for low birth weight, which justifies the heterogeneity of the data in this meta-analysis.

It is important to emphasize that the data for the meta-analysis considered breastfeeding present or absent. For a profound knowledge of this relationship, it would be interesting to observe the time of breastfeeding. From a nutritional, immunological and even affective development point of view, exclusive breastfeeding is known to be the ideal feeding for infants up to 6 months of age, which would be a protective factor against diseases and child malnutrition[58] and consequently of enamel defects. Some studies observed in a specific population with a high concentration of environmental pollutants such as PCDD/Fs (polychlorinated dibenzo-p-dioxins/dibenzofurans) and PCBs (Biphenyl Polychloride) that children with prolonged breastfeeding (longer than 8 months) had a higher frequency of enamel defects[59]. However, the same group of researchers in 2008 evaluated longitudinally the relation between frequency of MIH and the levels of PCDD/Fs and PCBs and the duration of the breastfeeding longitudinally.[50] Those authors observed that the levels of PCDD/Fs and PCBs and the duration of breastfeeding were not associated with the occurrence and severity of MIH. Thus, future studies are still required to evaluate this relationship (breastfeeding and MIH) in a deeper way in relation to the eating habits of children in early childhood.

When considering the postnatal period, respiratory problems and fever were associated with MIH. For respiratory problems, data was merged according to the variety of respiratory complications investigated by the studies, such as asthma, pneumonia, rhinitis, and breathing problems, which justify the heterogeneity of the data. The evaluation of fever was variable in the eligible studies. Questions or interviews with terms like “fever presence”, “high fever”, “fever during the first year of life”, “fever above 39 Celsius degrees”, “fever above 38.5 Celsius degrees”, “feverish state”, “frequent fever”, “high fever unexplained” were used to recall this exposure factor, but they are subjective to recall bias. Fever is commonly associated with childhood illnesses such as respiratory problems, explaining why both factors were associated with MIH.

We have not found association between infections during childhood and childhood illnesses with MIH. Data of infections were highly heterogeneous in this study. We observed that the studies evaluated a large variability of infection, such as tonsillitis, otitis, urinary tract infection, throat infection, gastroenteritis and oral infection. Most of the eligible studies also merge these infections, which prevented us from evaluating them separately to investigate their impact on MIH development. Similarly, childhood illness data was heterogeneous, and studies comprised several types of medical conditions, such as chickenpox, measles, varicella, rubella, mumps, scarlet fever, varicella zoster. The relation between childhood illnesses and MIH could be analyzed using a multiple statistical approach, once the use of medication as well as fever could be confounder variables. Although an earlier systematic review has concluded that early childhood health factors are associated with MIH, and childhood illnesses are among them, they did not present a meta-analysis of the available studies.[18]

Antibiotic use was not associated with MIH in the current systematic review. The role of drugs/medicine in the enamel formation may be evidenced in animal studies. It is noted that medicine can result in different effects on the development of the enamel. In a recent study, Munoz et al[54], evaluated whether antibiotics and non-steroidal anti-inflammatory drugs in childhood could disturb enamel mineralization in rats. Those authors observed that only acetaminophen and celecoxib showed a significant decrease in Ca and P when compared to the control samples. De Souza et al.[60] observed that amoxicillin reduced enamel thickness formation during the secretion. Thus, we can conclude that investigation about medication must be more specific in order to be associated with MIH.

This study showed that there are many studies focusing on MIH etiology, however, this subject needs an urgent improvement of the methodology of the data collection and multiple statistical approaches to test the systemic exposures related to MIH, since it seems to be a complex disease[3] with systemic and genetic interaction.[6, 31] Some final considerations about the quality of the evidence gathered by this systematic review are: According to the GRADE approach, the quality of the evidence produced by all meta-analyses was of very low quality; which means that there was very little evidence of the effect estimated and it may be substantially different from that measured. This review may encourage further well-delineated cohort studies to investigate important risk factors for MIH.

Conclusion

According to this systematic review, maternal illness, psychological stress, cesarean procedures, delivery complications, respiratory diseases, fever and childhood illnesses were significantly associated with MIH. However, as the evidence was gathered from observational studies with serious limitations in the risk of bias, imprecision and inconsistency, caution should be taken when interpreting these results.

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Table 1 – Electronic databases and search strategy**.**

Pubmed = 1843 retrieved articles	
#1((molar [MeSH Terms]) OR Tooth calcification [MeSH Terms]) OR dental enamel hypoplasia [MeSH Terms]) OR molar [Title/Abstract]) OR “Tooth calcification” [Title/Abstract]) OR "Dental enamel Hypoplasia" [Title/Abstract]) OR "Dental Hypoplasia" [Title/Abstract]) OR “first permanent molar” [Title/Abstract]) OR "molar incisor hypomineralization" [Title/Abstract]) OR "permanent first molars" [Title/Abstract]) OR "cheese molars" [Title/Abstract]) OR MIH [Title/Abstract]) OR "molar incisor hypomineralisation" [Title/Abstract]) OR "hypomineralized molars" [Title/Abstract]) OR "enamel opacities" [Title/Abstract]) OR "Enamel Defects" [Title/Abstract]) OR "Developmental Defects of Enamel" [Title/Abstract]) OR "Enamel Hypoplasia" [Title/Abstract]) OR "enamel hypomineralization" [Title/Abstract]) OR "Developmental dental defects" [Title/Abstract]) OR "Demarcated opacities" [Title/Abstract]))	#2((infant, low birth weight [MeSH Terms]) OR Hypoxia [MeSH Terms]) OR Apgar score [MeSH Terms]) OR premature birth [MeSH Terms]) OR Stress,Psychological [MeSH Terms]) OR pre-eclampsia [MeSH Terms]) OR Asphyxia neonatorum [MeSH Terms]) OR Respiration Disorders [MeSH Terms]) OR Caesarean section [MeSH Terms]) OR infections [MeSH Terms]) OR tonsillitis [MeSH Terms]) OR fever [MeSH Terms]) OR Jaundice, Neonatal [MeSH Terms]) OR otitis [MeSH Terms]) OR Hypoxia [Title/Abstract]) OR “Apgar score” [Title/Abstract]) OR “premature birth” [Title/Abstract]) OR “pre-eclampsia” [Title/Abstract]) OR “Asphyxia neonatorum” [Title/Abstract]) OR “Respiration Disorders” [Title/Abstract]) OR “Caesarean section” [Title/Abstract]) OR infections [Title/Abstract]) OR fever [Title/Abstract]) OR Jaundice [Title/Abstract]) OR “childhood illnesses” [Title/Abstract]) OR “birth prematurity” [Title/Abstract]) OR “birth complications” [Title/Abstract]) OR “Psychological stress” [Title/Abstract]) OR “maternal illness” [Title/Abstract]) OR “maternal alcohol” [Title/Abstract]) OR “maternal smoking” [Title/Abstract]) OR Caesarean [Title/Abstract]) OR “respiratory disease” [Title/Abstract])
#1 AND #2	

Table 2 -Summary of the studies selected for this systematic review.

Study ID	Study design	Patient's age mean±SD (years)	Number of patients [drop-outs]	Recruitment of patients	Methods to obtain data	Outcomes evaluated			Newcastle–Ottawa Scale		
						Associated factors	Prevalence of HMI Number (%)	Evaluation Criteria for MIH	S	C	O
Allazzam et al. 2014	Observational	8-12 [9.4 +- 1.3]	267 [n.r]	University	Parents' questionnaire	Prenatal exposure; perinatal exposure; early childhood illness	23 (8.6%)	EAPD 2003	***		**
Arrow.2009	Observational	6-9 [n.r]	634 (56.0%)	Schools	Parents' questionnaire and interview	Prenatal exposure; perinatal exposure; early childhood illness	n.r (n.r)	DDE index Modified	***		***
Beentjes and Weerheijm. 2002	Cross sectional	n.r [9.9 +-2.0]	45 [n.r]	n.r	Parents' questionnaire	Early childhood illness	24 (53.0%)	nr	*	*	**
Costa-silva et al.2013	Prospective	4-6 [n.r]	134 [n.r]	Schools	Parents' questionnaire	Prenatal exposure; perinatal exposure; early childhood illness	n.r (15.6%)	EAPD 2003	**		***
Dietrich et al. 2003	Case Control	10-17 [n.r]	2408 [0]	Schools	Parents' questionnaire	Perinatal exposure; early childhood illness	135 (5.6%)	DDE index Modified	*** *	*	**
Durmus et al. 2009	Case Control	7-14 [9.9 +-1.7]	228 [0]	University	Parents' questionnaire	Prenatal exposure; early childhood illness	n.r (24.0%)	EAPD 2003	**	*	**
Garot et al. 2016	Case Control	6-28 [14.3 +- 6.1]	849 (n.r)	Teaching Dental Hospital	Parents' questionnaire, medical records	Perinatal exposure	n.r(n.r)	EAPD 2003	***		**
Ghanin et al. 2013	Case Control	7 – 9 [n.r]	823 (82.3%)	Schools	Parents' questionnaire and interview	Prenatal exposure; perinatal exposure; early childhood illness	153 (18.6%)	EAPD 2003	*** *	*	***
Kim et al. 2016	Observational	8 – 9 [n.r]	950 [0]	Schools	Parents' questionnaire	Prenatal exposure; perinatal exposure; early childhood illness	n. r (7.1%)	EAPD 2003	*** *		**

Kuhnisch et al. 2015	coort	n.r [10.2 +- 0.2]	1048 [0]	Hospitals	Parents' questionnaire	Elevated Serum 25(OH) vitamina D	n.r (13.6%)	EAPD 2003	**		***
Kuhnisch et al. 2014	coort	10 [n.r] (10.2)	692 (84.4%)	Hospital GINI	Parents' questionnaire, medical records	Prenatal exposure; perinatal exposure; early childhood illness	428 (61.8%)	EAPD 2003	**		***
Kuscu et al. 2008	Observational	7 – 9 [n.r]	147 [0]	University	Parents' questionnaire and interview	Early childhood illness	n.r (14.9%)	EAPD 2003	*** *		**
Laisi et al. 2008	coort	7 – 10 (8.5 +-0.4)	167 [0]	Hospital	Parents' questionnaire, medical records	Duration of breast-feeding, concentration of PCDD/Fs or PCBs	24 (14.4%)	EAPD 2003	**		***
Laisi et al. 2009	Observational	7-12 (10.7 +- 1.3)	141 (52.2%)	Schools	Parents' questionnaire, medical records	Use of antibiotics during the first year of life	23 (16.3%)	EAPD 2003	***	*	***
Lima et al. 2015	Observational, Cross sectional	11 – 14 [n.r]	594 [0]	Schools	Parents' questionnaire	Prenatal exposure; perinatal exposure; early childhood illness	n.r (18.4%)	EAPD 2003	*** *		***
Loli et al. 2015	Case Control	6 – 13 (7.9 +1.7)	182 [0]	Pediatric Clinic	medical records	Aerosol Therapy	n.r (50.0%)	n.r	***	*	**
Lygidakis et al. 2008	Case Control	5-12 (8.1 +-1.3)	3518 [0]	Dental Clinic	Parents' questionnaire, medical records	Prenatal exposure; perinatal exposure; early childhood illness	360 (10.2%)	EAPD 2003	*** *	*	**
Mishra and Pandey 2016	Observational	8 – 12 [n.r]	1369 [0]	n.r	Parents' questionnaire	Early childhood illness	191 (13.9%)	n.r	**		*
Muratbegovic et al. 2007	Observational	12 [n.r]	560 [0]	Schools	Parents' questionnaire	Early childhood illness	69 (12.3%)	EAPD 2003	*** *	*	**
Pittiphat et al. 2014	Cross sectional	7 – 8 (8.0 +-0.5)	282 (67.1%)	Schools	Parents' questionnaire	Prenatal exposure; perinatal exposure; early childhood illness	78 (27.7%)	EAPD 2003	*** *	*	***
Rodrigues et al. 2016	Case Control	7 – 14 [n.r]	1179 (79.6%)	Schools	Parents' questionnaire	Prenatal exposure; perinatal exposure; early childhood illness	n.r (2.5%)	DDE index Modified	*** *	*	***

Sidaly et al. 2017	Case Control	8-10 [9.0 +- 0.8]	224 [0]	University Hospital of Olso	Parents' questionnaire	Apgar score	67 (23.5%)	EAPD 2003	****	**	***
Sonmez et al. 2013	Observational	7 – 12 [n.r] (9.5)	4049 (95.3%)	Schools	Parents' questionnaire	Prenatal exposure; perinatal exposure; early childhood illness	308 (7.7%)	EAPD 2003	****		***
Sousa et al. 2012	Observational	6 – 12 [n.r]	903 (68.67%)	Schools	Parents' questionnaire	Prenatal exposure; perinatal exposure; early childhood illness	n.r (24.9% rural area 17.8% urban area)	EAPD 2003	****		***
Sousa et al. 2013	Case control	7-12 (8.86 +1.28)	1151 (90.4%)	Schools	Parents' questionnaire	Prenatal exposure; perinatal exposure; early childhood illness	142 (12.3%)	EAPD 2003	****		***
Torino et al. 2016	Observational	8 – 9 [n.r]	1181 [0]	Schools	Parents' questionnaire	Prenatal exposure; perinatal exposure; early childhood illness	241 (20.4%)	EAPD 2003	****		**
Whatling and Fearne 2008	Case Control	6 – 13 [n.r] (8.7)	109 [0]	Hospital Royal London	Parents' questionnaire and interview	Prenatal exposure; perinatal exposure; early childhood illness	57 (52.3%)	n.r	****		**
Wuollet et al.2014	Coort	7-13 [10.0 +- 1.5]	818 (68.0%)	Schools	Parents' questionnaire	Prenatal exposure; perinatal exposure; early childhood illness	140 (17.1%)	EAPD 2003	***		***
Wuollet et al.2016	Coort	7- 12 [10.4 +- 1.3]	287 (80.0%)	Schools	medical records	Early childhood illness	11.5% HMI1 6.3% HMI2	EAPD 2003	***		***

ID – identification; SD – standard deviation; n.r. – not reported, MIH- molar incisor hypomineralization ; Academy of Pediatric Dentistry (EAPD): Demarcated opacity, posteruptive enamel breakdown, atypical restoration, extracted molar due to MIH, DDE Index Modified: Demarcated opacity, diffuse opacity, hypoplasia, other defects, demarcated opacity and hypoplasia, diffuse opacity and hypoplasia, all three defects together. The risk of bias used the Newcastle–Ottawa Scale: 0 to 9; patient selection (S)- 4 points for generalization and applicability was applied- up to 4 stars), comparability (C)of groups (2 points- up to 2 stars), exposure measurements (O) in cohort studies or case-control (3 points-up to 3 stars).

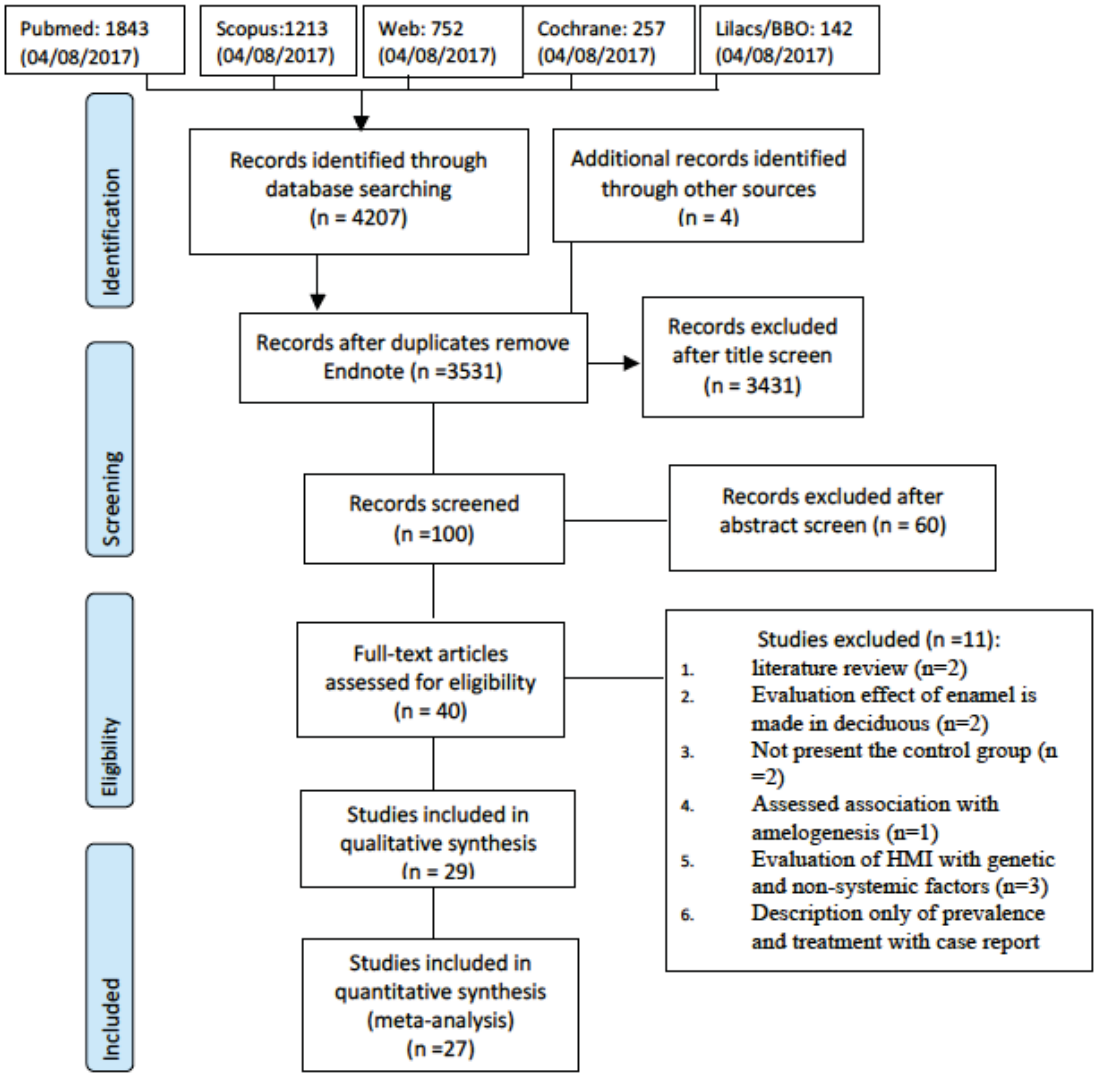

Figure 1 – Flow diagram of study

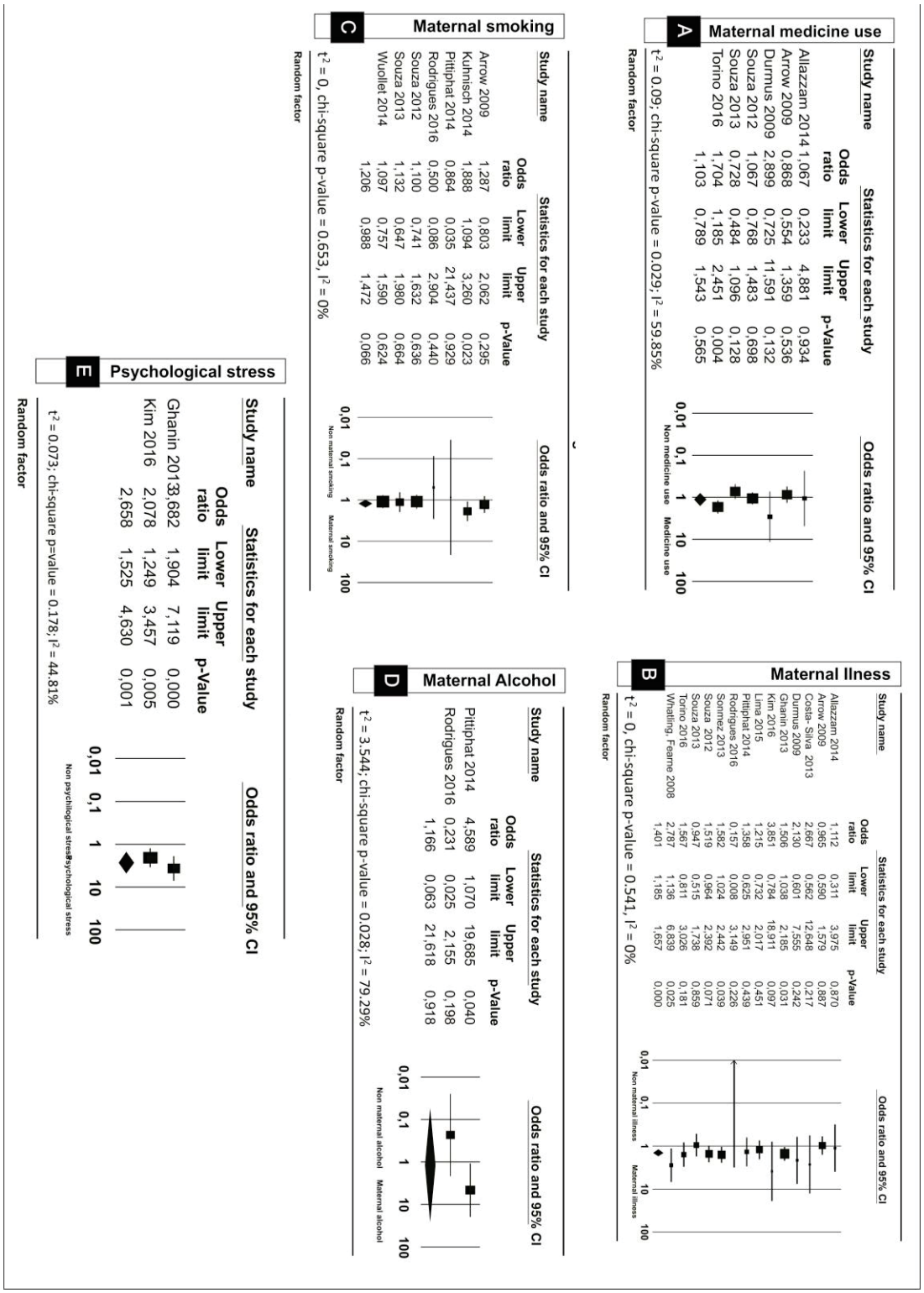

Figure 2 – Forest plots of exposures in the prenatal period (Maternal medicine use- A, maternal illnesses -B, maternal smoking- C, maternal alcohol use - D, psychological stress- E)

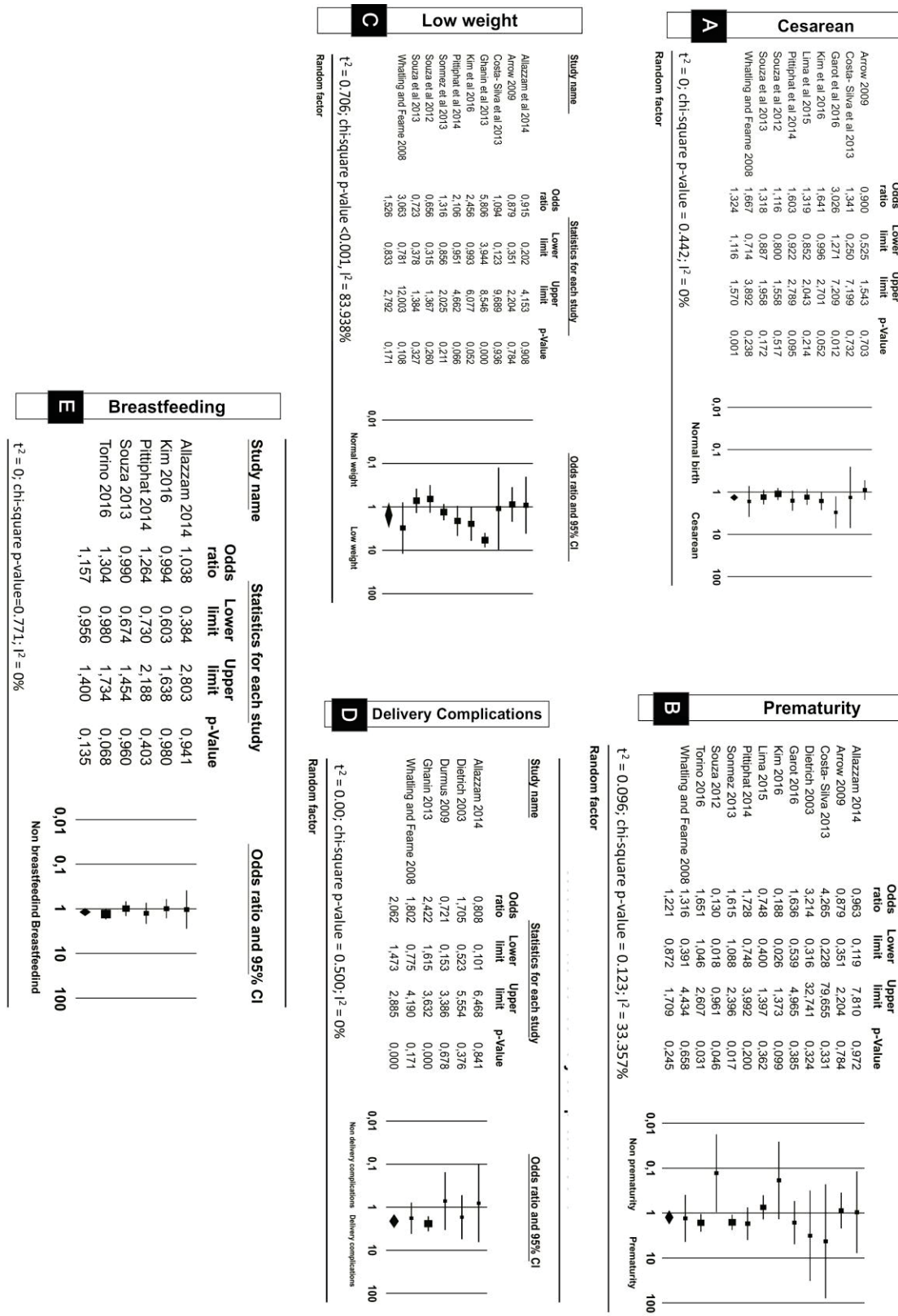

Figure 3- Forest plots of exposures in the perinatal period (caesarean delivery- A, prematurity- B, low birth weight- C, delivery complications- D, breastfeeding- E).

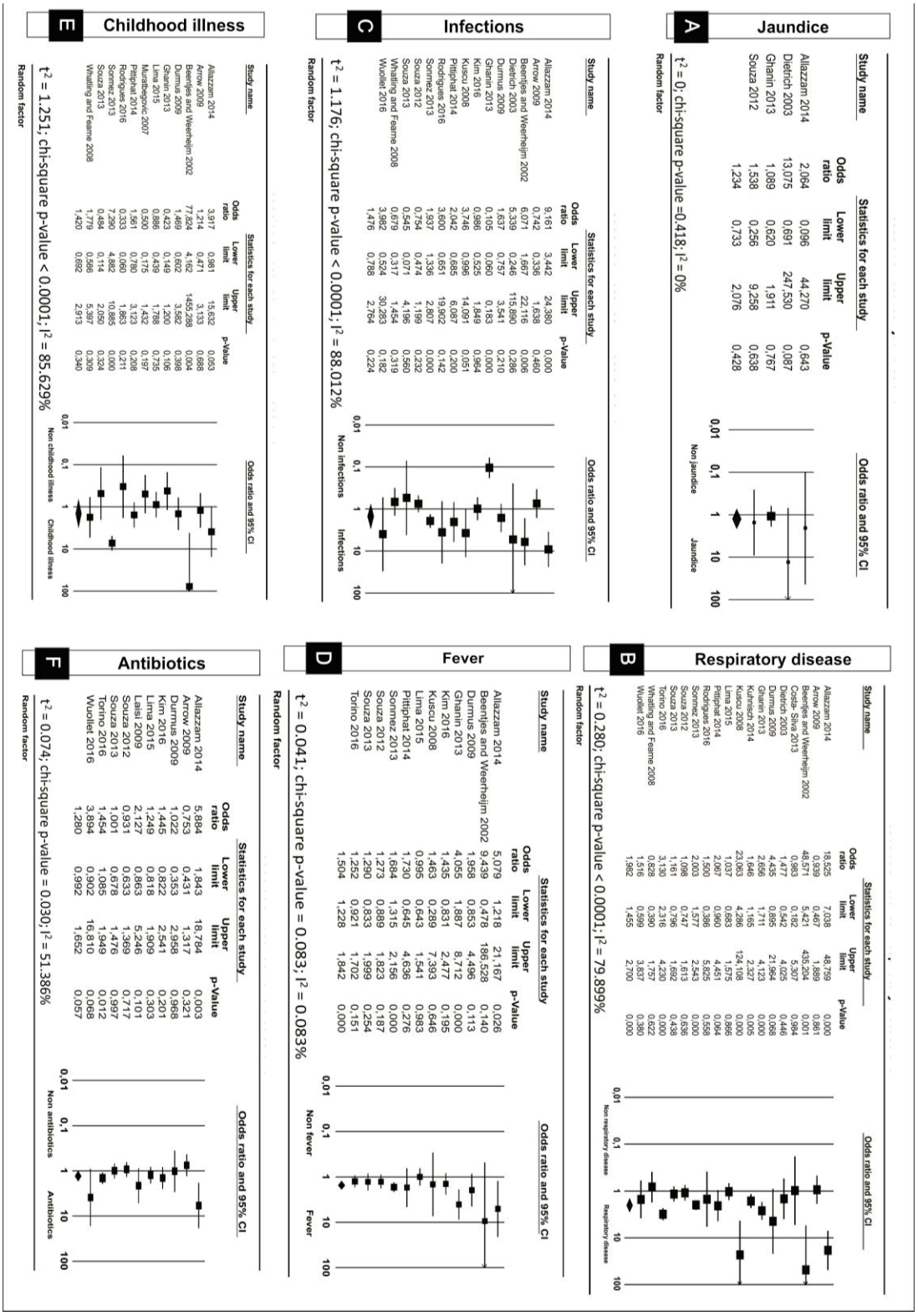

Figure 4- Forest plots of exposures in the postnatal period (jaundice- A, respiratory disease- B, infections C, fever- D, childhood illnesses- E and antibiotics- F).

4. ARTIGO 2

Systemic exposures are associated with hypomineralization of second primary molars. A hierarchical approach.

Esse artigo foi formatado de acordo com as normas do periódico International Journal of Paediatric dentistry.

Summary

Background: Hypomineralization of second primary molars (HSPM) is clinically represented by demarcated opacities in the enamel, involving from 1-4 second primary molars. The purpose of this cross-sectional study was to investigate if HSPM is associated with systemic exposures.

Design: A representative population-based sample of 731 school children aged 8 years old was randomly selected. Data on systemic exposures were collected by a structured questionnaire applied to the children's mothers. The HSPM was clinically assessed by calibrated examiners according to the modified-DDE index. Associations were analyzed by Poisson Multiple Regression considering a temporal hierarchical approach, evaluating the pre, peri and postnatal exposures.

Results: The prevalence of HSPM was 9.4% (n=69). Children whose mothers presented the use of tobacco (PR_a=2.44; p=0.001), the presence of hypertension (PR_a=1.73; p=0.044), the presence of complication during delivery (PR_a=1.83; p=0.032) and the occurrence of otitis during early childhood (PR_a=1.68; p=0.043) presented a higher prevalence of HSPM in the children.

Conclusion: The use of tobacco, the presence of hypertension, a complication during delivery and otitis during the first years of child`s life are associated with a higher prevalence of HSPM. Moreover, this data suggested that prenatal factors might have a greater influence on the development of HSPM.

Keywords: dental enamel hypoplasia; tooth, deciduous; etiology.

Introduction

Current literature shows that the etiology of demarcated hypomineralization is multifactorial and the possible factors could be occurring in synergism. The genetic, systemic and local factors have also been reported in some studies[1-3]. Since the enamel does not remodel, insults occurring during the enamel development process can generate permanent defects in the affected teeth. Hypoplasia is the quantitative loss of dental enamel and can be considered the most serious type among developmental defect of enamel (DDE), since it makes the tooth more susceptible to dental cavities, causing great sensitivity, the demarcated opacities, on the other hand, are qualitative losses of dental enamel, characterized by well-defined borders, slick surface and normal enamel thickness [4].

The presence of hypomineralization of second primary teeth (HSPM) has been indicated as a predictor of molar incisor hypomineralization (MIH)[5, 6.] This association could be explained due to the temporal coincidence between the mineralization of the first permanent molars and the second primary molars, with the last beginning to calcify the crown concomitantly around the 18th gestational week until 10 months of child's life[7]. Thus, exposure during the pre and perinatal periods could be associated with hypomineralization in not only second primary molars but also involving first permanent molars[8, 9]. It is supposed that the exposure associated with HSPM is also involved in the genesis of Molar-Incisor Hypomineralization[3, 7].

Although there are a lot of systemic factors related with DDE in primary teeth in the literature, there is little information about the systemic exposure associated with HSPM considering the hypothesis that HSPM is a predictor from MIH. Therefore, this study used the multiple model with a hierarchical approach to evaluating the exposures associated to HSPM, allowing the selection of independent variables more strongly associated to HSPM considering the different levels of association [10]. This approach allows the interpretation of the results based on the division of the independent variables in hierarchical blocks and is especially useful when there are multiple factors for a single outcome since it facilitates the evaluation of the influence of the independent variables and allows the identification of potential confounding factors [10]. According to our knowledge, there is no study in the literature that evaluates the systemic exposures associated with HSPM with a hierarchical approach, so this cross-sectional study is aimed to evaluate if systemic exposure, in the pre, peri and postnatal period, is associated with hypomineralization of second primary molars using a hierarchical multiple analysis.

Material and methods

Ethical approval

The cross-sectional study was approved by the Committee for Ethics in Research in Human Beings of the Health Sciences of the Federal University of Paraná (UFPR) (**1.613.829 / 2016)** and by the Municipal Department of Education. After the approval, the students and mothers were invited to participate in the study through an Informed Consent Term. This study was reported according to the STROBE statement for observational study (https://www.strobe-statement.org).

Elaboration of the questionnaire

Data on systemic exposures were collected by a structured questionnaire applied to the children's mothers, considering the prenatal, perinatal and postnatal periods.

The questionnaire addressed questions about the prenatal stage up to the child's third year of life, as described in the literature[11, 12], and was divided into three parts: prenatal, perinatal and postnatal history.

Calibration of the Examiners

Four examiners were trained and calibrated to diagnose DDE and MIH, using the index DDE-modified [4] which presents the following scores: demarcated opacity, diffuse opacity, and hypoplasia. For this purpose, 30 intraoral photographs of the DDE in primary teeth were selected, and the clinical situations associated with the differential diagnosis for the initial training. After that, 60 different photographs were selected with clinical situations involving all the different manifestations of DDE were analyzed independently by the examiners. After 1 week, the examiners (duplicate examination) independently analyzed the same photographs in a different order.

The results were compared with a gold standard and statistically analyzed using the kappa coefficient for intra-examiners and inter-examiners agreements. The objective of this step was to achieve agreements in parameters considered substantial ($K \geq 0.75$). The kappa for both inter and intra-examiners was ≥ 0.75. The kappa values according to the examiners and index are described in Frame 1.

Evaluation of the systemic factors

The evaluation of the systemic exposure was performed through a structured questionnaire applied to the child's mothers.

In the prenatal period, the following categories were divided: the presence of maternal malnutrition (malnutrition + anemia), the use of alcoholic drink, the use of tobacco, the use of illicit drugs, the use of antibiotics, the use of other medicine, the presence of diabetes, the

presence of fever, the presence of viruses, the presence of varicella and the presence of hypertension.

In the perinatal period, the categories were as follows: delivery complications, low birth weight dichotomized at ≤2.500 g and> 2.500 g, the use of infant incubator need, preterm birth was considered the birth before 37 weeks of gestation, twins; the presence of breastfeeding; and the duration of breastfeeding.

For the postnatal period the exposures were categorized into: the use of antibiotics, the use of other medicine, the presence of seizure, the presence of fever, the presence of otitis, the presence of urinary infection, the presence of throat infection, the presence of bronchitis, the presence of asthma, the presence of pneumonia, the presence of food intolerance (celiac disease + some food intolerance) and malnutrition (anemia + malnutrition).

Recruitment and eligibility criteria

Schoolchildren 8-years-old who had the Informed Consent properly signed by those parents was selected. Schoolchildren whose parents and/or schoolchildren who did not agree to participate in the study, who had orthodontic braces that would impair visualization, with syndromes associated with other types of defects of enamel or imperfect amelogenesis were excluded.

Sample size calculation

For the sample size calculation, data from the Brazilian Institute of Geography and Statistics in 2010 [13] and from the Education Department of the State of Paraná were used to calculate the population size. In order to estimate the representative sample size, the proportion of defects in enamel development in this population was set at 50%, the precision of 5%, the reference population of 143.701 students enrolled, a design effect factor of 1.8 to conglomerate the sample (in two stages, with the schools being raffled first and then classes), and the limit value of the rejection area of 1.96, 20% was added to compensate for eventual losses, resulting in the final sample size between 692 to 865 children. The sample was selected in accordance with nine sanitary districts of Curitiba.

The study participants were selected from the public schools in the city of Curitiba, located in the southern region of Brazil, with a population of 1.908.359 inhabitants and a Human Development Index (HDI) of 0.823, which is in 10th place on the Brazilian cities ranking according to UNDP (United Nations Development Program).

Twenty schools were randomly selected from the sanitary districts; new random sample draws were performed to select the schoolchildren classes. The random sequence and allocation concealment were generated by the software, which is freely available online

(www.randomizor.gov). An investigator who was not involved in the implementation of the study generated the random sequence.

Clinical Data Collection

The clinical data collection was carried out in a school environment by four calibrated examiners, using artificial light, dental mirror, dental probe blunt tip and sterile gauze. The data collection was carried out from November 2016 to September 2017.

Pilot study

A pilot study was conducted involving 80 schoolchildren enrolled in the municipal public-school system, at the same age as the main study sample. In this pilot, the clinical examination was performed under the same conditions and criteria established in the main study, as well as the questionnaires applied to the child' mothers were tested in order to evaluate the understanding and possible changes in the proposed methods. No changes were made to the questionnaire. Pilot study participants were not included in the final sample.

Statistical analysis of data

The dependent variable HSPM was categorized as "with HSPM" and "without HSPM"; the presence of this condition was computed when at least one-second primary molar was affected by demarcated opacity. For the prevalence of HSPM, the presence of at least one second primary molar with demarcated opacity according to the DDE-Modified index was considered [4].

The independent variables were categorized and analyzed descriptively. The family structure was dichotomized, according to the family nucleus, being classified as nucleated, when there was a stable relationship between those responsible, and non-nucleated, when the those responsible were single or widowed. The family income was dichotomized according to the median, in ≤ 2 Brazilian minimum wages (BMW) and > 2 BMW. The schooling of those responsible was dichotomized in ≤ 8 years and > 8 years. For ethnicity, the maternal report was considered.

For the analysis of the association between the HSPM (dependent variable categorized as "with HSPM" and "without HSPM") and systemic exposure, the univariate and multiple Poisson Regression analysis with robust variance was used with its respective prevalence ratio (PR), with a level of significance of 5%. The data was analyzed using Statistical Package for Social Sciences 16.0 for Windows (SPSS Inc., Chicago, Illinois, USA) and STATA 14.0 (StataCorp, Texas, USA).

To construct the multiple Poisson regression analysis, independent variables with $p<0.20$ in the association with HSPM were added. It was performed considering the

hierarchical approach, based on the conceptual and temporal framework (Figure 1) (10), in which the systemic exposure was divided from proximal to distal according to the period in which the exposure occurred.

Prenatal exposures (events that involved the mother's health during the pregnancy) were considered the distal block, thus the perinatal exposures were adjusted by prenatal and other variables of the intermediate block exposures whose $p<0.20$ in the univariate Poisson regression analysis. The postnatal exposures were considered the proximal block. Thus, it was adjusted by prenatal and perinatal exposures and by the other variables of the same block whose $p<0.20$ in the univariate and Poisson regression analysis (Figure 1). It is based on the framework that the prenatal exposure could determine the perinatal events as well as the postnatal comorbidities [10].

Results

From a potential eligible population of 865, a total of 784 pairs of children and mothers accepted to participate in the study (response rate 90.6%), from it 733 children were examined, 2 children were excluded from the study because they had some of the previously mentioned exclusion factors (use of orthodontic braces), resulting in a final sample of 731 children (Figure 2).

From this sample, 374 (51.16%) were males, the predominant ethnic group was Caucasian with 84.40% (617), the mean family income was US$ 579.07.

The prevalence of HSPM was 9.4% (CI 7.00– 12.00), with 4.2% of the tooth 55, 2.2% of the tooth 65; 4% of the tooth 75 and 4.5% of the tooth 85.

In the univariate analysis of the prenatal period, there existed a positive association between HSPM and the use of tobacco (PR = 2.60; CI 1.59 – 4.26; $p < 0.001$) and the presence of hypertension (PR = 2.01 CI 1.18 – 3.45; p = 0.010) (Table 2), but the multivariate model remained statistically associated with HSPM independently of the other variables: the use of tobacco (PR_a = 2.44; CI 1.47 – 4.06; p = 0.001), and the presence of hypertension (PR_a = 1.73; CI 1.01 – 2.95; p = 0.044), and delivery complication (PR_a = 1.83; CI 1.05 – 3.19; p = 0.032) (Table 3).

In the perinatal period, a positive association between HSPM and low birth weight (PR = 1.91; CI 1.00 – 3.64; p = 0.047), the need for an infant incubator (PR = 1.94; CI 1.05 – 3.60; p = 0.034) and twins (PR 3.11; CI 1.31 – 7.37; p = 0.010) (Table 2). There was not found any exposure during which time the perinatal period remained associated with HSPM when adjusted by prenatal variables (Table 3).

During the first years of a child`s life, the presence of otitis (PR = 1.90; CI 1.20 – 3.01; p = 0.006) and bronchitis (PR = 1.68; CI 1.00 – 2.82; p = 0.046) were associated with HSPM in the univariate analysis (Table 2), but only otitis (PR_a = 1.68; CI 1.01 – 2.79; p = 0.043) remained statistically significant associated with HSPM when adjusted by prenatal exposure (Table 3).

Discussion

According to our knowledge, this study is the first to use the multiple model with a hierarchical approach to evaluate exposures associated with HSPM. This allows the interpretation of the results based on the division of these independent variables into hierarchical blocks, which facilitates the evaluation of the influence of the several investigated exposures and the identification of possible confounding factors [10].

HSPM is a very common condition that affects children worldwide, its prevalence varies broadly, the prevalence of HSPM in this study was 9.4%. The rate in other previous studies was 4.9% in a research with 386 5-year-old children in the Netherlands [14]. The prevalence of 6.6% was found in Iraq with 809 children aged 7-9 years [7]. In India, the prevalence was 5.6% in a study with 978 children aged 6 to 8 years [5], 5.8 % in a study performed with 469 children aged 8 – 10 years in Nigeria [15], 14.5% in a research made in Spain with 414 children aged 8-9 years [8], and 14.1% in a study made with 623 children aged 3-5 years in Australia.

Children whose mothers presented tobacco use, hypertension and delivery complication presented a high prevalence of HSPM in the multivariate model. Our results agree with previous studies, in 2010, Vello et. al. [16] noted that maternal exposures such as the use of tobacco during pregnancy, early age, and pregnancy with multiple births are related to higher prevalence of enamel development defects. Elfrink et. al. in 2014 [17] found that maternal alcohol consumption during pregnancy is associated with deciduous molar hypomineralization. Considering nicotine as a toxin, a study in animals observed that the exposure to nicotine presented an alteration to the dental morphology, suggesting that biologically nicotine could affect the dental development [18].

In the perinatal period, a significant association in the univariate analysis with low birth weight, the need for an infant incubator, twins, complication in the perinatal period was found. However, when these exposures were adjusted by the prenatal exposure, none perinatal exposure was associated with HSPM. Thus, these results suggested that the prenatal exposure presented a greater impact on the HSPM than perinatal events in a temporal multiple approach. These results are different from Elfrink et. al. (2014) [17], that found an association

between primary molar hypomineralization and low birth weight. In a systematic review evaluating the DDE in premature infants, it was possible to observe an association between enamel opacities in primary teeth and birth weight <1500g as well as premature birth [19]. Wagner (2016) [20] in a study evaluating 377 3-year-old children found statistically significant associations between enamel development defects in children with preterm and low birth weight (OR = 4.90), children hospitalized in the first year of life (without prematurity / children with low birth weight) (OR = 4.44) and children with systemic antibiotic therapy (OR = 2.21). These different results could be explained by the differences between the studies. The studies investigated all types of developmental defects, including diffuse opacities or hypoplasia in all primary dentition, while our study investigated the factors specifically associated with HSPM. Another fundamental difference is related to the multiple model considering the hierarchical temporal approach.

In the present study, the postnatal exposure that was significantly associated with HSPM in the univariate analysis was the presence of otitis and bronchitis, both are related to respiratory or infection problems. When adjusted by the hierarchical multiple, children with otitis presented 68% more prevalence of HSPM than children who not suffered otitis. There is no data about this association in the literature. It is important to emphasize that otitis is an infectious condition, which could co-exist with the presence of fever and use of medicines. However, these exposures were not associated with HSPM in this study. Elfrink et. al. in 2014 [17] in a cohort study with 6690 children noted that any fever in the first year of the child's life is associated with the primary molar hypomineralization. Wagner in 2016 [20] observed that HSPM was associated with hospitalization and antibiotics in the first year of life. Biologically, experiments in animals showed that fever, exposure to dioxin and use of antibiotics (especially amoxicillin) disturbed the enamel formation, consequently, they may be associated with enamel hypomineralization [18, 21-24]. The postnatal systemic conditions should be seen in a holistic approach.

Negre-Barber et al. (2016) [8] observed that children with HSPM presented a positive likelihood ratio of 10.3 for MIH, which means that the probability of MIH is 10.3 times higher in those with HSPM than children not affected by it. While the negative likelihood ratio of 0.57 indicates a lower ability to predict the absence of MIH in children without HSPM. Based on the association between MIH and HSPM, it has been postulated that a similar set of causal factors (premature birth, low birth weight and/or poor gestational health) may play a role in the development of HSPM and MIH, once there is an overlapping period of first permanent molar and second primary molar mineralization [7, 17, 25]. Calcification of the

second primary molars begins in the 4th month of fetal life and the complete formation in the 36th month of child' life. This is somewhat earlier than the development of the first permanent molars and incisors, but the developmental periods of the second primary molars and the first permanent molars overlap [15]. If a risk factor occurs during this period of overlap, hypomineralization may occur in both primary and permanent dentition [26]. Thus, the presence of HSPM may serve as a useful predictor for MIH[5], which is extremely important from a preventative point of view so that family counseling can begin and provide preventive planning for these children

The etiology of HSPM, as well as MIH, are still unclear and understood, due to the low evidence of the retrospective study. However, they are defined as a complex disease, in which systemic and ambient exposure, adding a genetic predisposition (genetic polymorphisms), have been associated with the development of the defect [9]. Alaluusua (2010)[27], in a review study about the etiological exposure of MIH, suggested that the combined effect of several factors should also be taken into account. Our hypothesis is that this combined effect occurs according to a chronological period of amelogenesis by increments, which could explain why the demarcated opacities are asymmetric in different areas of the dental enamel crown. Thus, the exposure could be associated considering a hierarchical temporal of amelogenesis (Figure 3)

A limitation of the present study was the transversal design, which presents bias in researching the retrospective exposures, such as memory bias and possible confounding factors that we cannot isolate, and the lack of a validated questionnaire to assess systemic exposures However, the present study presented a new view of the role of systemic events in enamel development considering the hierarchical approach at the time of the exposure.

Conclusion

Based on this study, it is possible to conclude that use of tobacco, hypertension, a complication in delivery during the pregnancy and otitis during the first years of a child`s life can be associated with HSPM. Moreover, it is possible to conclude that prenatal factors have a greater influence on the development of HSPM.

Bullet points

The diagnosis of HSPM allows an early diagnosis of MIH, since the second deciduous molar is present in the mouth 4 years before the permanent molar, this allows a preventive planning.

It allows the identification, through a systematized anamnesis, of groups at risk for hypomineralization of dental enamel.

The association between the use of tobacco, hypertension, a complication in delivery during the pregnancy and otitis during the first years of a child`s life and HSPM.

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Frame 1 – Kappa agreements according the examiners of the indexes.

Examiners	DDE intra	DDE inter
Examiner 1	0.93	1.00
Examiner 2	0.80	0.75
Examiner 3	0.88	0.89
Examiner 4	0.80	0.86

Note: the inter examiners kappa values were evaluated with gold standard examiner (JFS)

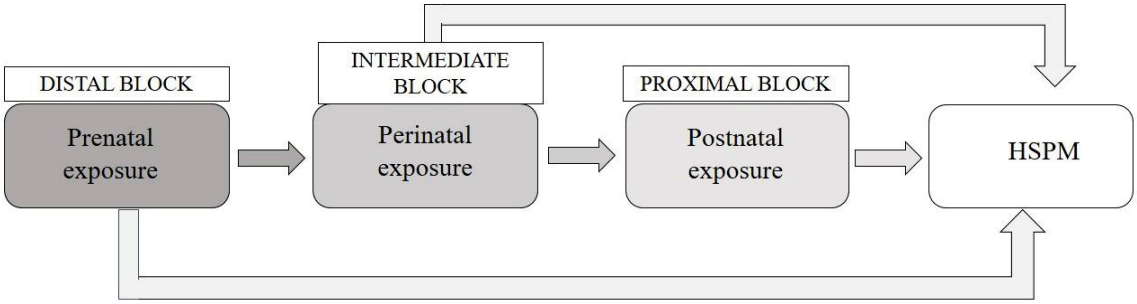

Figure 1: Conceptual hierarchical framework of risk factors for HSPM adapted from Victora et al (1997) [10].

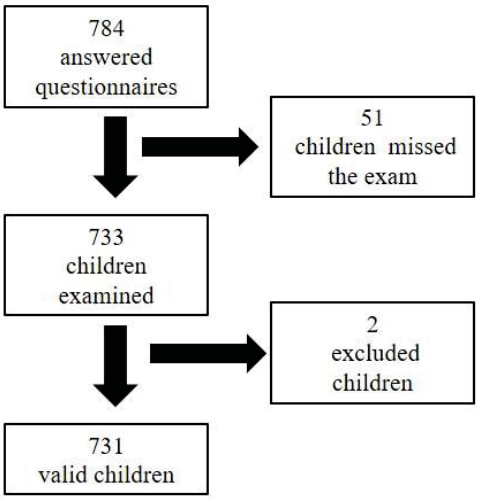

Figure 2. Fluxogram of the participants.

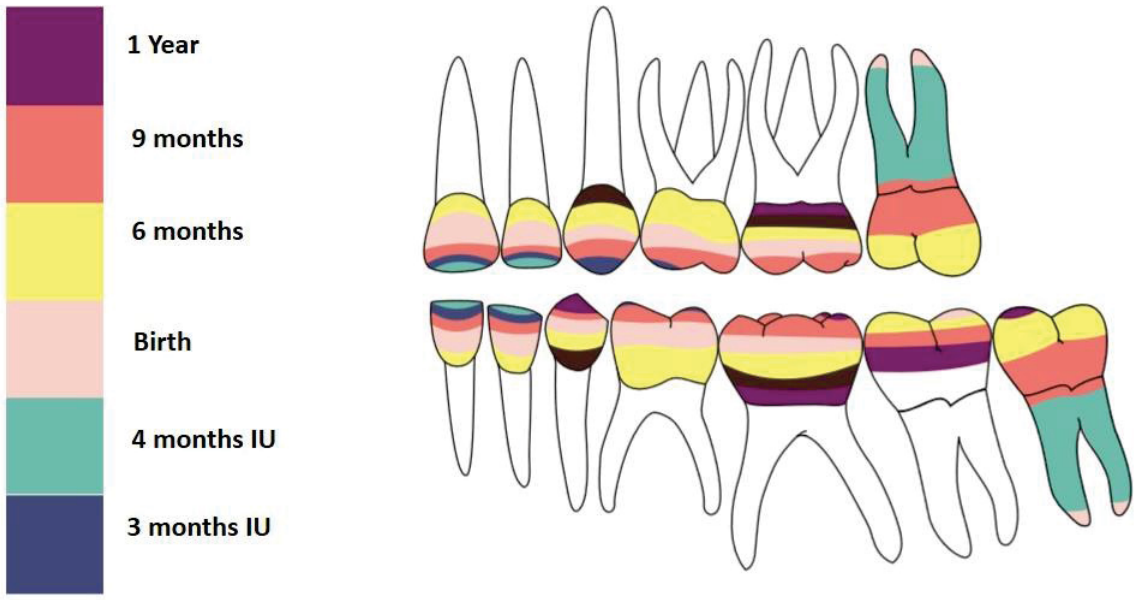

Figure 3: chronology of the formation of primary teeth and 1st permanent molar based on Goran et. al. and Irurita et.al. [28, 29]

Table 1 - Socioeconomic characteristics of 8-year-old schoolchildren in the city of Curitiba – 2017 (n=731)

Variables	Categories	n (%)
Gender	Male	374(51.6)
	Female	357 (48.84)
Ethnicity	Caucasian	617(84.40)
	Afro-descendant	89(12.18)
	Asiatic	11 (1.50)
	Indian	14 (1.92)
Family BMW	> two wages	258 (35.29)
	≤ two wages	473(64.71)
Schooling	> 8 years of study	518 (71.65)
	≤ 8 years of study	205 (28.35)
Number of residents at home	< 4	487 (66.62)
	≥ 5	244 (33.38)
Family structure	Nucleated family	501 (69.58)
	Non - nucleated family	219(30.42)
Labor activity	At home	250(35.8)
	Out of home	449(64.2)
Marital status	Single	134(18.6)
	Divorced	75(10.4)
	Widower	10(1.4)
	Married / Stable Relationship	501(69.6)

Note: BMW: Brazilian minimum wage.

Table 2 – Univariate Poisson regression model - Systemic exposures associated with Hypomineralization of Second Primary molars (HSPM).

Pre Natal Period						
Exposure		**HSPM**		**Total**	**PRC (CI 95%)**	**p value**
		Yes N (%)	**No N (%)**			
Maternal Nutrition	Yes	29 (12.1)	211(87.9)	240	1.34 (0.83 – 2.15)	0.220
	No	33 (9.0)	334 (91.0)	367	1.00	
Alcoholic drink	Yes	2 (6.9)	27 (93.1)	29	1.36 (0.35 – 5.30)	0.655
	No	63 (9.4)	607 (90.6)	670	1.00	
Tobacco	Yes	18 (20.5)	70 (79.5)	88	2.60 (1.59 – 4.26)	**<0.001**
	No	48 (7.9)	563 (92.1)	611	1.00	
Illicit drugs	Yes	1 (11.1)	8 (88.9)	9	1.21 (0.18 – 7.82)	0.838
	No	63 (9.1)	626 (90.9)	689	1.00	
Antibiotics	Yes	2 (14.3)	12 (85.7)	14	1.05 (0.87 – 1.26)	0.607
	No	67 (9.4)	643 (90.6)	710	1.00	
Other medicines	Yes	10 (9.8)	92 (90.2)	102	1.04 (0.54 – 1.98)	0.899
	No	52 (9.4)	501 (90.6)	553	1.00	
Diabetes	Yes	2 (6.7)	28 (93.3)	30	1.40 (0.36 – 5.46)	0.626
	No	62 (9.4)	601 (90.6)	663	1.00	
Fever	Yes	6 (11.1)	48 (88.9)	54	1.16 (0.52 – 2.57)	0.710
	No	56 (9.6)	530 (90.4)	586	1.00	
Virus	Yes	9 (9.3)	88 (90.7)	97	1.06 (0.54 – 2.08)	0.853
	No	53 (9.9)	483 (90.1)	536	1.00	
Hypertension	Yes	15 (16.3)	77 (83.7)	92	2.01 (1.18 – 3.45)	**0.010**
	No	48 (8.1)	546 (91.9)	594	1.00	
Chickenpox	Yes	13 (14.6)	76 (85.4)	89	1.66 (0.94 – 2.93)	0.076
	No	52 (8.8)	542 (91.2)	594	1.00	
Perinatal Period						
Exposure		**HSPM**		**Total**	**PRC (CI 95%)**	**p Value**
		Yes N (%)	**No N (%)**			
Delivery complication	Yes	14 (21.2)	52 (78.8)	66	2,55 (1.49 – 4.35)	**0.001**
	No	52 (8.3)	574 (91.7)	626	1.00	
Low Birth Weight	Yes	9 (17.0)	44 (83.0)	53	1.91 (1.00 – 3.64)	**0.047**
	No	60 (8.8)	618 (91.2)	678	1.00	
Infant incubator	Yes	10 (17.2)	48 (82.8)	58	1,94 (1.05 – 3.60)	**0.034**
	No	57 (8.9)	586 (91.1)	643	1.00	
Prematurity	Yes	9 (12.5)	63 (87.5)	72	1.20 (0.50 – 2.87)	0.679
	No	56 (9.0)	566 (91.0)	622	1.00	
Twins	Yes	4 (28.6)	10 (71.4)	14	3.11 (1.31 – 7.37)	**0.010**
	No	63 (9.2)	624 (90.8)	687	1.00	
Breastfeeding	Yes	43 (9.5)	412 (90.5)	455	0.98 (0.89 – 1.08)	0.701
	No	5 (11.4)	39 (88.6)	44	1.00	
Duration of breastfeeding	≤12 months	28 (8,9)	284 (91,1)	312	1.15 (0.66 – 2.01)	0.606
	>12 months	19 (10,4)	164 (89,6)	183	1.00	
Post Natal Period						
Exposure		**HSPM**		**Total**	**PRC (CI 95%)**	**p Value**
		Yes N (%)	**No N (%)**			
Other medicines	Yes	16 (11.4)	124 (88.6)	140	1.29 (0.74 – 2.24)	0.357
	No	39 (8.8)	403 (91.2)	442	1.00	
Antibiotics	Yes	52 (10.7)	433 (89.3)	485	1.45 (0.79 – 2.65)	0.221
	No	12 (7.4)	151 (92.6)	163	1.00	
Seizure	Yes	2 (11.1)	16 (88.9)	18	1.14 (0.30 – 4.32)	0.840
	No	66 (9.7)	615 (90.3)	681	1.00	
Fever	Yes	23 (10.5)	196 (89.5)	219	1.18 (0.72 – 1.92)	0.496
	No	40 (8.9)	411 (91.1)	451	1.00	
Otitis	Yes	29 (14.5)	171 (85.5)	200	1.90 (1.20 – 3.01)	**0.006**

	No	37 (7.6)	450 (92.4)	487	1.00	
Throat infection	Yes	49 (10.6)	414 (89.4)	463	1.39 (0.82 – 2.36)	0.217
	No	17 (7.6)	207 (92.4)	224	1.00	
Urinary infection	Yes	9 (9.4)	87 (90.6)	96	1.07 (0.55 – 2.08)	0.838
	No	60 (10.1)	537 (89.9)	597	1.00	
Bronchitis	Yes	17 (14.4)	101 (85.4)	118	1.68 (1.00 – 2.82)	**0.046**
	No	49 (8.5)	525 (91.5)	574	1.00	
Asthma	Yes	10 (15.6)	54 (84.4)	64	1.73 (0.93 – 3.21)	0.084
	No	57 (9.0)	574 (91.0)	631	1.00	
Pneumonia	Yes	8 (10.4)	69 (89.6)	77	1.08 (0.54 – 2.18)	0.816
	No	59 (9.6)	558 (90.4)	617	1.00	
Food Intolerance	Yes	1 (4.2)	23 (95.8)	24	0.42 (0.06 – 2.90)	0.381
	No	66 (9.9)	601 (90.1)	667	1.00	
Malnutrition	Yes	4 (6.7)	56 (93.3)	60	0.65 (0.24 – 1.74)	0.399
	No	64 (10.1)	567 (89.9)	631	1.00	

Note: PR_c: Crude prevalence ratio calculated by the univariate Poisson regression $p<0.05$.

Table 3 – Multiple Poisson regression model considering the hierarchical approach according to the pre, peri and postnatal periods.

Exposure		**HSPM**	
		PRa (CI 95%)	**P Value**
Prenatal period – Distal block			
Tobacco	Yes	2.44 (1.47 – 4.06)	0.001
	No	1	
Delivery Complications	Yes	1.83 (1.05 – 3.19)	0.032
	No	1	
Hypertension	Yes	1.73 (1.01 – 2.95)	0.044
	No	1	
Perinatal period* - Intermediate block			
Twins	Yes	2.17 (0.84-2.72)	0.129
	No	1.00	
Infant incubator	Yes	1.51 (0.84 – 2.72)	0.165
	No	1	
Low Birth Weight	Yes	1.70 (0.86 – 3.34)	0.122
	No	1.00	
Postnatal period* - Proximal block			
Otitis	Yes	1.68 (1.01 – 2.79)	0.043
	No	1.00	
Bronchitis	Yes	1.30 (0.64 – 2.61)	0.455
	No	1.00	
Asthma	Yes	1.43 (0.61 – 3.36)	0.402
	No	1.00	

Note: PR_a: Adjusted prevalence ratio calculated by the multiple Poisson regression $p<0.05$.

5. ARTIGO 3

Polymorphism in Vitamin D- receptor (VDR) and enamel hypomineralization. A cross-sectional population based study

Esse artigo foi formatado de acordo com as normas do periódico Oral Disease.

ABSTRACT

Molar-Incisor hypomineralization (MIH) and hypomineralized second primary molars (HSPM) are specifics developmental defect of enamel, which affect, respectively, first permanent molars and second primary molars. The purpose of this cross-sectional study was to investigate if polymorphisms in VDR gene are associated with the prevalence of MIH or HSPM. A representative population-based sample of 731 schoolchildren aged 8 years old was randomly selected in Curitiba-PR. The socioeconomic and demographic information were obtained by a structured questionnaire applied for child's mothers. The MIH, HSPM was assessed clinically by four calibrated examiners (Kappa> 0.80) using the EAPD criteria (2003), modified-DDE index (1992) respectively. The rsVDR739837 and rsVDR2228570 markers (VDR marker for vitamin D) were genotyped using the real-time PCR technique. The genotypes were categorized as additive genotype, dominant allele and recessive allele. Associations were analyzed by Poisson Regression with robust variance (α=0.05). There are no association between MIH/ HSPM with markers rs739837 and rs2228570. However, individuals with allele GT/GG on the marker VDR rs739837 presented higher prevalence of MIH in the incisor teeth (PR = 2.34; IC 1.08 – 5.07; p = 0.03). It is possible to conclude that the presence of MIH as well as the presence of HSPM are not associated with polymorphisms on the rs739837 and rs2228570 markers. But, the presence of MIH in incisors are associated to polymorphisms in the rs739837 marker. Observational studies in other populations are required to verify the association of genetic factors with MIH and HSPM.

Key – words: dental enamel hypoplasia; genetic polymorphism

INTRODUCTION

Enamel hypomineralization is defined as qualitative dental enamel defects, in which the enamel presents less mineral, porous structure resulting in alteration on the mechanical resistance and higher risk to dental caries (1-3). Molar-Incisor hypomineralization (MIH) and hypomineralized second primary molars (HSPM) are specific developmental defect of enamel, which affect, respectively, first permanent molars and second primary molars. In 2001, the European Academy of Paediatric Dentistry (EAPD) defined MIH as a defect of dental enamel with systemic origin that affects at least one permanent molar and often permanent incisors (4). In 2008, Elfrink et al (2008) defined HSPM as a demarcated hypomineralization that affects 1 to 4 second primary molars (5). Studies have been considering HSPM as a predictor for MIH, once children with HSPM have 4 to 6 times higher chance to have MIH (6).

Regarding to the etiological aspects, several factors could be associated to MIH and HSPM during the prenatal life until first childhood (7).The etiology of MIH and HSPM remain uncertain (8). It could be considered as complex conditions, in which systemic factors in synergism with genetic factors may be involved in the hypomineralization origin (9-11). While some authors considered only as a genetic condition (12).

Vitamin D is intimately involved in tooth formation (13), it is not only a regulator of mineral homeostasis that maintains a stable relationship between phosphate and calcium ions, which may influence the quality of bone, enamel and dentin, it is also involved in the individual's immune response.

Vitamin D deficiency leads to uncontrolled changes in the immune system, which blocks the correct immune response to oral microbiota (14, 15). The main function of vitamin D is to maintain plasma calcium concentrations at a constant level, which is important for healthy bone development as well as healthy tooth development.

Vitamin D stimulates the mineralization of dental enamel by binding to receptors, which are expressed in both the dental and bone cells (16). As ameloblasts and odontoblasts are target cells of vitamin D it is plausible to state that vitamin D deficiency is linked to enamel development disorders (17). Animal studies have shown that disruption of the vitamin D pathway leads to inadequate levels of calcium and phosphorus in circulating plasma, resulting in decreased bone mineralization and a negative impact on tooth mineralization (18).

The vitamin D receptor (VDR) is considered as a mediator for the effect of vitamin D-related bio mineralization (19). VDR controls the transcription of several target genes, many

of which are expressed by ameloblasts and odontoblasts, poor functioning of the vitamin D pathway may result from vitamin D deficiency or from the VDR gene mutation (13).

The gene VDR is located on chromosome 12q13.11 and contains several polymorphic regions (14). The VDR gene has the effect of modulating the biological function of the major vitamin D metabolites, therefore, it plays an important role in the formation of teeth, particularly in the calcification of dentin enamel (15).The polymorphisms of the VDR gene are important factors for the normal development of the enamel, it is known that variations in the human genome of the VDR can be associated to hereditary tooth enamel malformations (19).

Since the inadequate level of vitamin D may induce defects in dentin and enamel during dental development (20), the hypothesis of this study is that polymorphisms in VDR gene increase the prevalence of MIH and HSPM.

MATERIALS AND METHODS

Ethical approval

This cross-sectional study was approved by the Committee for Ethics in Research in Human Beings of the Health Sciences of the Federal University of Paraná (UFPR) (1.613.829 / 2016) and by the Municipal Department of Education. After the approval, the students and mothers were invited to participate in the study through an Informed Consent Term. It was reported according the STROBE statements (https://www.strobe-statement.org).

Calibration of the Examiners

Four examiners were trained and calibrated to diagnose DDE and MIH, using the index DDE-modified (21) and the criteria for MIH form European Academy of Paediatric Dentistry (4), which presents the following scores for DDE: demarcated opacity, diffuse opacity, hypoplasia and for MIH: demarcated opacity (white, yellow or brown), post eruptive fracture (involving enamel or enamel and dentin), atypical restoration (satisfactory or unsatisfactory) and exodontia by MIH. For this purpose, 30 intraoral photographs of the developmental defects of enamel in primary teeth were selected, and the clinical situations associated to the differential diagnosis for the initial training. After that, 60 different photographs were selected with clinical situations involving all the different manifestations of DDE and MIH were analyzed independently by the examiners. After 1 week, the examiners (duplicate examination) analyzed independently the same photographs in a different order.

The results were compared with a gold standard and statistically analyzed using the kappa coefficient for intra-examiners and inter-examiners agreements. The objective of this step was to achieve agreements in parameters considered substantial ($K \geq 0.75$. The kappa for

booth inter and intra-examiners were >0.75. The kappa values according the examiners and index are described on Frame 1.

Recruitment and eligibility criteria

It was selected 8-year-old schoolchildren who had the Informed Consent properly signed by those parents, with the first permanent and incisor irrupted. It was excluded the schoolchildren who had orthodontic braces that would impair visualization, with syndromes associated with other types of defects of enamel or imperfect amelogenesis

Sample size calculation

For the sample size calculation, data from the Brazilian Institute of Geography and Statistics in 2010 (22) and from the Education Department of the State of Paraná were used to calculate the population size. In order to estimate the representative sample size, the proportion of defects in enamel development in this population was set at 50%, the precision of 5%, the reference population of 143.701 students enrolled, a design effect factor of 1.8 to conglomerate sample (in two stages, with the schools being raffled first and then classes), and the limit value of the rejection area of 1.96, 20% were added to compensate for eventual losses, resulting in the final sample size between 692 to 865 children. The sample were stratified according to nine sanitary districts of Curitiba.

The study participants were selected from the public schools in the city of Curitiba, located in the southern region of Brazil, with a population of 1.908.359 inhabitants and a Human Development Index (HDI) of 0.823, which are in 10th place on the Brazilian cities ranking according to UNDP (United Nations Development Program)

Twenty schools were selected randomly from the sanitary districts (strata); new random sample draws were performed to select the schoolchildren. The random sequence and allocation concealment was generated by the software, which is freely available online (www.randomizor.gov). An investigator who was not involved in the implementation of the study generated the random sequence.

Pilot study

A pilot study was conducted involving 80 schoolchildren enrolled in the municipal public-school system, at the same age as the main study sample. In this pilot, the clinical examination was performed under the same conditions and criteria established in the main study, as well as the questionnaires applied to the child' mothers were tested in order to evaluate the understanding and possible changes in the proposed methods. Pilot study participants were not included in the sample. None change was performed form the pilot study.

Clinical Data Collection

The clinical data collection was carried out in a school environment by previously trained and calibrated examiners four, with the assistance of artificial light, dental mirror, dental probe blunt tip and sterile gauze. The data collection carried out from November 2016 to September 2017.

Genomic polymorphisms

DNA was obtained from epithelial cells of the oral mucosa after a mouthwash of 5 ml of 3% glucose solution for 2 min, and then scraping the oral mucosa with sterilized wooden spatula (23),and stored in a thermal box during transportation until then purified with 10 M ammonium and 1 mM EDTA (24). The 15 ml Falcon tubes containing the epithelial cells were centrifuged for 10 minutes at 3000 rpm at the temperature environment with the function of sedimentary the cells and oral debris. The supernatant was discarded immediately after centrifugation to prevent cells and epithelial structures (Pellet) from the bottom of the tube. For the second wash it was1300 µl of TE extraction buffer (10 mM TRIS, 5 mM EDTA, 0.5% SDS) in each vial, and frozen in sequence. In order to perform the DNA extraction, two steps, on different days, were needed. On the first day, the samples were completely thawed and followed by pipetting 10 µl of proteinase K (Sigma Chemical Co., St. Louis, MO, USA) (20mg / ml) in each tube. After mixing, the tubes were kept in a 55 ° C water bath during the night. After incubation, the mixture was transferred to a 2 ml eppendorf and pipet 500 µl of ammonium acetate (8 M in 1 mM EDTA), followed by vortex at high speed for 5 minutes, and thereafter, centrifuged at 13000 rpm for 16 min. Then the content was divided into two eppendorfs of 1.5 ml, being 900 µl in each, and added with 540 µl of isopropanol (isopropyl alcohol). Samples were inverted delta twenty times and after centrifugation for 7 min at 13,000 rpm. The supernatant was suspended at the bottom of the tube and isopropanol was discarded. 1 ml of 70% ethanol in each tube which was inverted several times to wash the DNA pellet and centrifuged at 7 min at 13.000 rpm. Then the ethanol was discarded and allowed to dry at room temperature for approximately 4 hours. When the tube was 50 µl of TE buffer (10 mM TRIS, 1 mM EDTA, pH 7.76) for suspension of the DNA and left at room temperature for 24 hours, then left in a refrigerator for two days and stored at -20 ° C. The amount of DNA and the purity of each sample was determined by Spectrophotometry (NanoDrop 1000, Thermo Fisher Scientific, US). The DNA concentration was obtained by readings at 260 nm. In order to estimate the purity, the proportion used was 260 nm / 280 nm. Samples were diluted with TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) at a concentration of 4 ng / µl. In each well of the plate was pipetted 1,5 µl diluted DNA plus the reaction

containing 1.5 µl of Master Mix, 0.1 µl of 40X SNP and covered with an optical adhesive film (MicroAmp Optical Adhesive Film, Thermo Fisher Scientific, MA, USA). The 96-well plates were placed in the thermal cycler (GeneAmp ™ PCR System 9700, Thermo Fisher Scientific, MA, USA) at 95 ° C for 10 minutes and 40 cycles of 92 ° C for 15 seconds.

The gene of choice was the VDR located on the long arm of chromosome 12, band 13, sub band 11.

The genetic markers VDR *rs2228570* and VDR *rs739837* were used for genotyping using the real-time PCR technique. The selected marker data can be viewed in frame 2.

Statistical analysis of data

The dependent variables were considered HSPM, MIH, MIH in incisors which were categorized as "presence" or "absence". The presence of MIH was computed according to the EAPD (2003), as at least one first permanent molar affected by the demarcated hypomineralization. The presence of HSPM was computed according as at least one second primary molar affected by the demarcated hypomineralization. The genotypes were categorized as additive genotype, dominant allele or recessive allele. For the association between MIH and VDR genotypes, Poisson regression with robust variance analysis were calculated. The level of significance adopted was 0.05. The independent variables were categorized and analyzed descriptively. The family structure was dichotomized, according to the family nucleus, being classified as nucleated, when there was a stable relationship between those responsible, and non-nucleated, when the responsible were single or widowed. The family income was dichotomized according the median, in ≤ 2 Brazilian minimum wage (BMW) and> 2 BMW. The schooling of those responsible was dichotomized in ≤ 8 years and> 8 years. For ethnicity, the maternal report was considered. The category of lower prevalence was considered the reference. The statistical analyses were performed using Statistical Package for Social Sciences 16.0 for Windows (SPSS Inc., Chicago, Illinois, USA) and STATA 14.0 (Statacorp, Texas, USA).

RESULTS

From a potential eligible population of 865, a total of 784 pairs of children and mothers accepted to participated in the study (response rate 90.6%), from it 733 children were examined, 2 children were excluded from the study because they had some of the previously mentioned exclusion factors (use of orthodontic braces), resulting in a final sample of 731 children (Figure 1).

The sample was composed by 374 (51.16%) males, the predominant ethnic group was Caucasian with 84.40% (617), the mean family income was US$ 579.07.

It was found no significant association between MIH and the markers *rs739837* and *rs2228570*, however when an association with the presence of MIH in the incisor teeth was evaluated, a significant association was observed with the recessive allele of the marker VDR 739837 and with the GT allele in the additive genotype model of the marker VDR 739837. (Table 2)

DISCUSSION

Based on the premise that vitamin D stimulates enamel mineralization through receptors expressed in dental cells (16), and that enamel-forming proteins expressed in ameloblasts and odontoblasts are dependent on vitamin D, investigation of the association of developmental defects enamel with polymorphisms in the vitamin D gene is valid (26). Furthermore, vitamin D resistance is known to be caused primarily by deficiency or polymorphisms in the VDR gene (27), and a mutation in vitamin D receptors may result in resistance to the normal effects of vitamin D, which may compromise dental formation (27). This study tested the hypothesis that polymorphisms in the VDR gene may be associated with MIH and HSPM in a population sample. The data from the present study showed that genetic variations in the VDR gene are not associated with the prevalence of MIH and HSPM.

Interestingly, when presence of MIH in incisors was analyzed, it was observed that children with G allele (GT / GG) in the marker *rs739837* showed a higher prevalence of MIH in incisors. The criteria for MIH diagnosis requires the presence of at least one first molar with demarcated opacity (4), and the incisors are frequently affected. It is known that the lesions of hypomineralization affect the molars more severely, and that the higher the number of impacted molars, the greater the chance of lesions on incisors (28). Thus, the association between polymorphism in the *rs739837* marker with MIH in incisors can be explained by the fact that these children who present in incisors may present a more serious (severe) phenotype of hypomineralization that commonly affects only the first permanent molars, having a prevalence of incisors involved as increases the number of molars affected by hypomineralization (28).

Berdal in 1993 in an animal study observed in the incisors of rats a physiological alteration in the activity of odontoblasts in animals that had vitamin D deficiency, since genes responsive to vitamin D encode important proteins in the formation of mineralized dental tissues (29). The inadequate level of vitamin D in the individual has also been reported associated with enamel hypoplasia, and with an increase in the incidence of dental caries (20).

Rummens in 2003 in a study with animals showed in an experiment with rats that fetuses of mothers who had an interrupted VDR gene had a defective bone and dental

mineralization (30). Similarly, Descroix in 2010 observed that vitamin D deficient rats had several dental alterations, with morphogenesis and abnormal cell differentiation (13).

Zhang in 2009, also in a study carried out with mice observed the different regulation of vitamin D pathways in mineral deposition in the lower incisors of VDR knockout mice, noting that dentin presented a lower mineral density with multiple radiolucent spots, dentin maturation in mice deficient in VDR was delayed and disorganized, indicating that the mineralization and maturation of dentin is compromised by VDR deficiency.

Still in the same study, using micro CT scan to measure the mineral deposition of enamel in dentin, it was possible to observe in VDR mice - / - hypomineralization of the enamel in the apical region of the incisors, confirming through the MEV an early maturation of the enamel in the corresponding region, the pattern of distribution of the enamel and dentin mineral deposition in the VDR - / - mice being significantly different from the VDR + +, indicating that VDR deficiency affects both enamel and dentin (31).

The relationship between vitamin D and tooth formation can be explained by the fact that VDR controls the transcription of several target genes expressed by ameloblasts and odontoblasts. (13). Although the mechanisms that VDR deficiency influences odontoblasts are still unclear, it is known that VDR is expressed in odontoblasts and ameloblasts, which in turn are target cells for vitamin D function.

Based on this, one study suggested that 1,25 (OH) D works by regulating VDR, which induces structural gene products, including calcium binding proteins and several important cell matrix proteins in dentin formation (18).

Investigations of serum concentrations of 1,25 (OH) D showed a correlation between higher serum concentrations of 1,25 (OH) D (measured by blood sample) and lower prevalence of molar and incisor hypomineralization and dental caries in children aged 10 years old in Germany in a retrospective 10-year follow-up study (17). It can be concluded that 1,25 (OH) D can act specifically on enamel and dentin formation through a complex network of genomic pathways. (32)

Among the limitations of the present study, there is the factor of not obtaining data on the serum concentrations of vitamin D during the process of formation of the evaluated teeth because it is a retrospective information and limited to memory bias. It is believed that vitamin D levels may act as a confounding factor between the HMI / HSPM relationship with polymorphisms in the VDR gene.

According to our knowledge, this is the first population-based study evaluating polymorphisms in the VDR gene with MIH / HSPM. Thus, it is suggested that future

investigations on factors associated with demarcated hypomineralizations such as MIH and HSMP also include information on vitamin D levels and markers of the VDR gene.

CONCLUSION

Based on these results, it can be concluded that genetic polymorphisms in the VDR gene were not associated with the presence of MIH as well as the presence of HSPM, but an association between the presence of MIH in incisors with polymorphisms in the *rs7398* marker was observed. Regarding to the multifactorial origin of MIH and HSPM, the present data suggested that genetic polymorphisms in the VDR gene could be involved in severe phenotypes (MIH in incisors) of demarcated opacities. Future observational studies population based are necessary to verify the association of polymorphisms in the VDR gene with MIH and HSPM.

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Frame 1 – Kappa agreements according the examiners to the indexes.

Examiners	EAPD intra	EAPD inter	DDE intra	DDE inter
Examiner 1	0.92	0.92	0.93	1.00
Examiner 2	0.79	0.92	0.80	0.75
Examiner 3	0.80	0.90	0.88	0.89
Examiner 4	0.75	0.94	0.80	0.86

Note: the inter examiners kappa values was evaluated with gold standard examiner

Frame 2 – candidate gene and its polymorphisms

Gene	Polymorphism	Locus	Change of base	Minimum allele frequency
VDR	*rs 2228570*	47879112	A/C/G/T	0.328
	rs739837	47844438	C/G/T	0.494

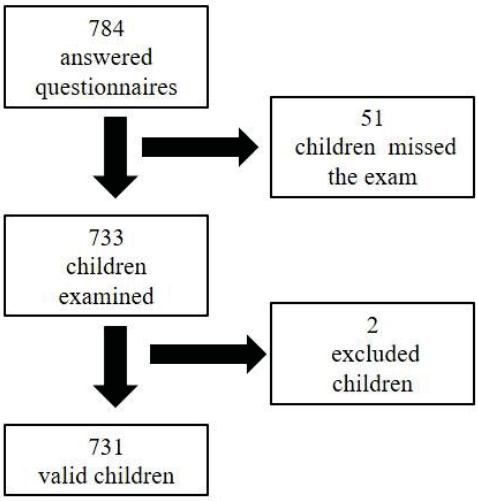

Figure 1. Fluxogram of the participants.

Table 1 - Socioeconomic characteristics of study population (Curitiba, Brazil, 2017)

Variables	Categories	n (%)
Gender	Male	374(51.6)
	Female	357 (48.84)
Ethnicity	Caucasian	617(84.40)
	Afro-descendant	89 (12.18)
	Asiatic	11 (1.50)
	Indian	14 (1.92)
Family BMW	> two wages	258 (35.29)
	≤ two wages	473(64.71)
Schooling	> 8 years of study	518 (71.65)
	≤ 8 years of study	205 (28.35)
	< 4	487 (66.62)

Number of residents at home	≥ 5	244 (33.38)
Family structure	Nucleated family	501 (69.58)
	Non - nucleated family	219(30.42)
Labor	At home	250(35.8)
	Out of home	449(64.2)
Marital status	Single	134(18.6)
	Divorced	75(10.4)
	Widower	10(1.4)
	Married / Stable Relationship	501(69.6)

Table 2. Prevalence ratio (PR_c) of MIH, HSPM according to the genotype, dominant and recessive models of VDR genes.

Genotype		**MIH**		**Total N**	**PR_c (IC95%)**	**P value**
		Presence n(%)	**Absence n(%)**			
739837 genotype	TT	23 (23.0)	77 (77.0)	100	Reference	
	GT	119 (72.56)	45 (27.44)	164	1.19 (0.77–1.84)	0.42
	GG	58 (76.32)	18 (23.68)	76	1.02 (0.59–1.76)	0.91
739837 dominant	TT/GT	68 (25.76)	196 (74.24)	264	1	0.71
	GG	18 (23.68)	58 (76.32)	76	0.91 (0.58–1.44)	
739837 recessive	GG/GT	63 (26.25)	177 (73.75)	240	1	0.53
	TT	23 (23.0)	77 (77.0)	100	1.14 (0.75–1.73)	
2228570 genotype	AA	5 (14.3)	30 (85.7)	35	Reference	
	AG	19 (12.5)	133 (87.5)	152	0.87 (0.35–.18)	0.77
	GG	22 (14.3)	132 (85.7)	154	1.00 (0.40-2.46)	1.00
2228570 dominant	AG/AA	24 (12.8)	163 (87.2)	187	1	0.69
	GG	22 (14.3)	132 (85.7)	154	1.11 (0.64–1.90)	
2228570 recessive	AG/GG	41 (13.4)	265 (86.6)	306	0.93 (0.39–2.21)	0.88
	AA	5 (14.3)	30 (85.7)	35	1	

Genotype		**MIH in incisors**		**Total N**	**PR_c (IC95%)**	**P value**
		Presence n(%)	**Absence n(%)**			
739837 genotype	TT	7 (7.1)	92 (92.9)	99	Reference	
	GT	27 (17.0)	132 (83.0)	159	2.40 (1.08–5.31)	**0.03**
	GG	12 (15.8)	64 (84.2)	76	2.23 (0.92–5.40)	0.07
739837 dominant	TT/GT	34 (13.2)	224 (86.8)	258	1	0.56
	GG	12 (15.8)	64 (84.2)	76	1.19 (0.65–2.19)	
739837 recessive	GG/GT	39 (16.6)	196 (83.4)	235	2.34 (1.08–5.07)	**0.03**
	TT	7 (7.1)	92 (92.9)	99	1	
2228570 genotype	AA	2 (5.7)	33 (94.3)	35	Reference	
	AG	10 (6.8)	138 (93.2)	148	1.18 (0.27-5.16)	0.82
	GG	11 (7.2)	142 (92.8)	153	1.25 (0.29-5.53)	0.75
2228570 dominant	AG/AA	12 (6.6)	171 (93.4)	183	1	0.82
	GG	11 (7.2)	142 (92.8)	153	1.09 (0.49–2.41)	
2228570 recessive	AG/GG	21 (7.0)	280 (93.0)	301	1.22 (0.29–4.99)	0.78
	AA	2 (5.7)	33 (94.3)	35		

Genotype		**HSPM**		**Total N**	**PR (IC95%)**	**P value**
		Presence n(%)	**Absence n(%)**			
739837 genotype	TT	13 (13.13)	86 (86.87)	99	Reference	
	GT	17 (10.69)	142 (89.31)	159	0.81 (0.41–1.60)	0.55
	GG	7 (9.21)	69 (90.79)	76	0.70 (0.29–1.67)	0.42
739837	TT/GT	30 (11.63)	228 (88.37)	258	1	0.56

dominant	GG	7 (9.21)	69 (90.79)	76	0.81 (0.40–1.63)	
739837	GG/GT	24 (10.21)	211 (89.79)	235	0.77 (0.41–1.46)	0.43
recessive	TT	13 (13.13)	86 (86.87)	99	1	
2228570	AA	6 (17.1)	29 (82.9)	35	Reference	
genotype	AG	13 (8.8)	135 (91.2)	148	0.51 (0.21–.25)	0.14
	GG	18 (11.8)	135 (88.2)	153	0.68 (0.29–1.60)	0.38
2228570	AA/AG	19 (10.4)	164 (89.6)	183	1	0.68
dominant	GG	18 (11.8)	135 (88.2)	153	1.13 (0.61–.08)	
2228570	AG/GG	31 (10.3)	270 (82.9)	301	0.60 (0.26–1.34)	0.21
recessive	AA	6 (17.1)	29 (82.9)	35	1	

Note:Prevalence rate (PR_c) calculated by Poisson regression analysis. Level of significance 0.05.

6. CONSIDERAÇÕES FINAIS

Considerando que a HSMD têm sido apresentada como preditora para a HMI, e que ambas são clinicamente similares, acredita-se que podem apresentar uma etiologia coincidente. Assim, com base nos resultados obtidos na revisão sistemática com meta-análise podemos concluir que a HMI está associada às doenças maternas e ao estresse psicológico durante a gestação, bem como parto do tipo cesárea, complicações no parto, assim como a presença de doenças respiratórias, febre na infância.

Quanto à HSMD, baseado no estudo epidemiológico observacional encontrou-se que o uso de cigarros durante a gestação, presença de hipertensão materna no período gestacional, complicações no parto e otite até os 3 primeiros anos de vida aumentaram a prevalência de HSMD.

Considerando ambos os defeitos HMI e HSMD em relação à polimorfismo no gene VDR, notou-se que quando computado apenas a presença da HMI e HSMD não houve associação com polimorfismos em ambos os marcadores investigados. Porém, quando analisado a presença da HMI em incisivos observou-se associação entre a presença de HMI em incisivos com polimorfismos no marcador *rs7398*.

Diante desses estudos, sugere-se que tanto a HMI como a HSMD são associadas às exposições sistêmicas, mas também podem ter um componente genético, caracterizando-as como condições de etiologia complexa. A associação encontrada entre polimorfismo no marcador *rs7398* com HMI em incisivos pode sugerir que há a participação de outras proteínas, diferentes daquelas relacionadas à matriz do esmalte, mas que são importantes para regulação e/ou desenvolvimento do esmalte. Sugere-se que estudos prospectivos são necessários para avaliar as exposições sistêmicas e genéticas envolvidas na HMI e HSMD.

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8. APÊNDICES

APÊNDICE 1 – TLCE

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Nós, Juliana Feltrin de Souza e Magdalena Raquel Torres Reys da Universidade Federal do Paraná, estamos convidando **VOCÊ pai/mãe responsável e SEU (SUA) FILHO** escolares matriculados nas escolas estaduais de Curitiba a participar de um estudo intitulado **"Hipomineralização Molar-Incisivo em uma população sul-brasileira: Prevalência, fatores sistêmicos associados e polimorfismos genéticos"**. Este estudo é importante para que possamos avaliar alterações no esmalte dentário, cárie dentária e suas possíveis causas.

1. O objetivo desta pesquisa é observar a frequência de crianças afetadas pela Hipomineralização (defeito no esmalte dentário), cárie dentária e avaliar as possíveis causas.
2. Caso você participe da pesquisa, será necessário preencher um questionário, que será enviado a sua casa e seu(sua) filho(a) participará de um exame bucal realizado no ambiente escolar, onde seu filho(a) estuda, em que será realizado também a coleta de saliva do mesmo, para avaliar as possíveis causas genéticas dos defeitos de esmalte. O exame bucal e coleta de saliva terão a duração entre 5 e 10 minutos. Para tanto você deverá enviar o questionário preenchido à escolar do seu filho.
3. O exame bucal é como qualquer exame odontológico de rotina, mas o exame não terá nenhuma consequência para seu filho(a). Se seu filho(a) mostrar-se contrário(a) à realização do exame ou constrangido(a), este será interrompido imediatamente. Se você apresentar algum constrangimento ao preencher o questionário, mesmo preenchendo em sua casa, poderá deixar a resposta em branco. O uso das amostras de saliva serão unicamente para essa pesquisa e salienta que logo após essa análise, as amostras de saliva serão descartadas de forma apropriada.
4. Dentre os benefícios diretos da pesquisa, caso de seu(sua) filho(a) apresentar alguma alteração na boca que indique tratamento, você será informado(a) e orientado(a) a levar seu(sua) filho(a) para buscar atendimento nas clínicas de Odontologia da Universidade Federal do Paraná.
5. A sua participação e de seu (sua) filho(a) neste estudo é voluntária, e se você não quiser mais fazer parte da pesquisa poderá desistir a qualquer momento. As informações relacionadas ao estudo poderão ser conhecidas por pessoas autorizadas (professora orientadora e aluna). O material obtido – questionários e ficha do exame clínico odontológico– será utilizado unicamente para esta pesquisa e será incinerado após 03 anos do término do estudo. As amostras de saliva serão descartadas de forma apropriada como material biológico logo após a análise.
6. Os pesquisadores responsáveis por este estudo poderão ser localizados no Campus Botânico da Universidade Federal do Paraná (Av. Prefeito Lothário Meissner, 632 - Jardim Botânico, Curitiba/PR, CEP 80210-170), no Departamento de Estomatologia ou pelo e-mail: julianafeltrin@hotmail.com e/ou magdalentorres@hotmail.es, para esclarecer eventuais dúvidas que você possa ter e fornecer-lhe as informações que queira, antes, durante ou depois de encerrado o estudo. Se você tiver dúvidas sobre seus direitos como participante de pesquisa, você pode contatar também o Comitê de Ética em Pesquisa em Seres Humanos (CEP/SD) do Setor de Ciências da Saúde da Universidade Federal do Paraná, pelo telefone (41)3360-7259.

Eu,_____, li esse Termo de Consentimento e compreendi a natureza e objetivo do estudo do qual EU concordei em participar. Eu também concordo com a participação DO(A) MEU FILHO(A) _____ neste estudo. A explicação que recebi menciona os riscos e benefícios. Eu entendi que sou livre para interromper MINHA participação e a participação do(a) MEU FILHO(A) a qualquer momento, sem justificar minha decisão e sem qualquer prejuízo para mim nem para meu filho(a). EU concordo voluntariamente em participar deste estudo e consinto a participação voluntária do(a) meu FILHO(A).

Curitiba, ____ de _____ de _____.

_____ _____

Assinatura do pai / responsável Assinatura do pesquisador responsável

Responsável legal pelo participante da pesquisa _____
Pesquisador responsável ou quem aplicou o TCLE _____
Orientador:

Comitê de Ética em Pesquisa com Seres Humanos do Setor de Ciências da Saúde da UFPR | CEP/SD Rua Padre Camargo, 285 | térreo | Alto da Glória | Curitiba/PR | CEP 80060-240 | cometica.saude@ufpr.br - telefone (041) 3360-7259

APENDICE 2 – Questionário eventos sistêmicos

Para completar os dados da pesquisa, precisamos que você responda às questões abaixo. **Não existe resposta certa ou errada, mas a resposta verdadeira é muito importante para que se conheça a realidade das crianças** (marque X).

Nome da criança:_____

Data de nascimento da criança:____/___/___

Qual o gênero da Criança? ( ) Feminino ( ) Masculino

Qual a etnia da Criança? ( ) Branca ( ) Negra ( ) Amarela ( ) Indígena ( ) Parda

Qual o seu nome:_____**Qual é a sua idade?** _____

Qual o seu endereço:_____**CEP**_____**Qual o bairro?**_____

Qual o seu telefone para contato? _____

- **Qual a sua relação com a criança?**

( ) Sou a mãe
( ) Sou o pai
( ) Outro: Qual?_____

- **Qual o seu estado civil?**

( ) Solteira(o)
( ) Separada(o)
( ) Viúva(o)
( ) Casada(o) ou relação estável (Morando junto há 5 anos)

- **A criança mora com você?**

( ) Sim
( ) Não

- **Quantas pessoas moram na mesma casa da criança (número total de moradores)?** _____

- **Qual é a renda mensal da sua casa?**

R$_____(reais)
(Incluir o total da casa: salários mínimos, Bolsa Família, Seguro desemprego e "bicos" de todos os moradores da sua casa.)

<u>Vamos falar um pouco sobre VOCÊ:</u>

- **VOCÊ trabalha**:

( ) Em casa/Aposentado ( ) Fora de casa

- **VOCÊ estudou até qual série?**

( ) Não estudou
( ) Primário(1ª a 4ª série) incompleto
( ) Primário (1ª a 4ª série) completo
( ) Ginasial (5ª a 8ª série) incompleto
( ) Ginasial (5ª a 8ª série) completo
( ) Colegial (ensino médio) incompleto
( ) Colegial (ensino médio) completo
( ) Superior (faculdade) incompleto
( ) Superior (faculdade) completo

- **Mãe/ pai ou responsável, quando foi a SUA última visita ao dentista?**

( ) Há menos de 1 mês
( ) De 1 a 6 meses
( ) De 6 meses a 1 ano
( ) Há mais de 1 ano
( ) Nunca foi
( ) Não lembra

- **Por que VOCÊ procurou o dentista pela última vez?**

( ) Para consulta preventiva
( ) Para resolver algum problema ou dor
( ) Nunca foi ao dentista

- **Como você considera a situação da saúde de SUA boca e de seus dentes?**

( ) Boa
( ) Razoável
( ) Ruim

- **VOCÊ teve dor de dentes nos últimos 6 meses?**

( ) Sim
( ) Não

- **Se VOCÊ tiver que ir ao dentista amanhã, como você se sentiria?**

( ) Eu estaria esperando uma experiência razoavelmente agradável
( ) Eu não me importaria
( ) Eu me sentiria ligeiramente desconfortável
( ) Eu acho que eu me sentiria desconfortável e teria dor
( ) Eu estaria com muito medo do que o dentista me faria

- **Quando VOCÊ está esperando na sala de espera do dentista, como você se sente?**

( ) Relaxado
( ) Meio desconfortável
( ) Tenso
( ) Ansioso
( ) Tão ansioso que começo a suar ou começo a me sentir mal

- **Quando VOCÊ está na cadeira odontológica esperando o dentista preparar o motor para trabalhar nos seus dentes, como você se sente?**

( ) Relaxado
( ) Meio desconfortável
( ) Tenso
( ) Ansioso
( ) Tão ansioso que começo a suar a me sentir mau

- **Você está na cadeira odontológica. Enquanto você aguarda o dentista pegar os instrumentos para raspar os seus dentes (perto da gengiva), como você se sente?**

( ) Relaxado
( ) Meio desconfortável
( ) Tenso
( ) Ansioso
( ) Tão ansioso que começo a suar ou começo a me sentir mal

Agora vamos falar um pouco sobre seu Filho(a)

- **Seu FILHO(A) já foi ao dentista alguma vez**?

( ) Sim
( ) Não
( ) Não Lembro

- **Se sim, onde?**

( ) Serviço público
( ) Serviço particular
( ) Serviço Público e também no Particular
( ) Nunca foi ao dentista

- **Você acha que o seu FILHO(A) tem medo de ir ao dentista?**

( ) Não tem medo
( ) Um pouco de medo
( ) Tem medo
( ) Sim, muito medo

- **O que você acha da saúde bucal do seu FILHO(A)?**

( ) Ruim
( ) Razoável
( ) Boa
( ) Não sei

- **Quantas vezes seu FILHO(A) escova os dentes por dia?**

( ) Nenhuma
( ) Uma
( ) Duas
( ) Mais de duas

- **O seu FILHO(A) já teve dor de dente?**

( ) Sim
( ) Não
( ) Não Lembro

- **Você fez alguma coisa para o aliviar a dor de dente do seu FILHO(A)?**

( ) Não
( ) Sim, dei um remédio que tinha em casa
( ) Sim, levei ao dentista
( ) Nunca teve dor de dente

- **Seu FILHO(A) já faltou à escola / CMEI porque estava com dor de dente?**

( ) Sim
( ) Não
( ) Não Lembro

- **O que VOCÊ já parou de fazer para estar com seu filho(a) que estava com dor de dente, que você considera mais relevante?**

( ) Faltei ao trabalho
( ) Parei de fazer tarefas domésticas
( ) Deixei de cuidar de outra criança
( ) Outras atividades
( ) Meu filho(a) nunca teve dor de dente

- **Quando foi a ultima vez que seu filho (a) foi ao dentista?**

( ) Menos de 1 ano
( ) Entre 1 e 3 anos
( ) Mais que 3 anos
( ) Nunca foi

- **Nessa última consulta por qual motivo você levou seu filho (a) ao dentista?**

( ) Para consulta preventiva
( ) Para resolver algum problema ou dor
( ) Nunca foi ao dentista
( ) Não sei ou não Lembro

- **Alguma vez você precisou de algum tratamento odontológico e não teve como pagar por este tratamento ou não conseguiu vaga para este atendimento na rede pública?**

( ) Sim
( ) Não
( ) Não Lembro

Queremos saber sobre a história médica do seu filho(a). Tente recordar se durante a GESTAÇÃO a Mãe da criança teve alguns desses problemas:

- **Episódios Múltiplos de Febre alta?**

( ) Sim
( ) Não
( ) Não Lembro

- **Virose?**

( ) Sim
( ) Não
( ) Não Lembro

- **Tomou remédios por tempo prolongado?**

( ) Sim, qual?_____
( ) Não
( ) Não Lembro

- **Diabetes?**

( ) Sim
( ) Não
( ) Não Lembro

- **Vômitos Prolongados?**

( ) Sim
( ) Não
( ) Não Lembro

- **Má nutrição?**

( ) Sim
( ) Não
( ) Não Lembro

- **Anemia?**

( ) Sim
( ) Não
( ) Não Lembro

- **Varicela (catapora)?**

( ) Sim
( ) Não
( ) Não Lembro

- **Pressão alta?**

( ) Sim
( ) Não
( ) Não Lembro

- **Consumo de álcool?**

( ) Sim
( ) Não
( ) Não Lembro

- **Consumo de cigarro?**

( ) Sim
( ) Não
( ) Não Lembro

- **Consumo de drogas Ilícitas?**

( ) Sim
( ) Não
( ) Não Lembro

- **Outros problemas ?**

( ) Não
( ) Sim, Qual_____

<u>Tente recordar sobre o PARTO, do seu filho(a)</u>

- **O parto de seu filho(a) foi**

( ) Natural
( ) Cesariana

- **Foi necessário usar fórceps?**

( ) Sim
( ) Não

- **O parto foi prolongado?**

( ) Sim, Quantas horas?_____
( ) Não

- **Houveram complicações no parto?**

( ) Sim, Qual? _____
( ) Não

- **Foram Gêmeos?**

( ) Sim
( ) Não

- **O Nascimento foi prematuro?**

( ) Sim, Quantas semanas?_____
( ) Não

- **Seu filho nasceu com qual peso?**

Nasceu com: _____
( ) Não Lembro

- **Outros problemas ?**

( ) Não
( ) Sim, Qual_____

<u>Tente recordar sobre o PRIMEIRO MÊS DE VIDA seu filho(a) teve alguns desses problemas</u>

- **Teve que ficar na Incubadora?**

( ) Sim, Quantos dias?_____
( ) Não
( ) Não Lembro

- **Problemas cardíacos?**

( ) Sim
( ) Não
( ) Não Lembro

- **Icterícia (amarelão)?**

( ) Sim
( ) Não
( ) Não Lembro

- **Diabetes?**

( ) Sim
( ) Não
( ) Não Lembro

- **Vômito Prolongado?**

( ) Sim
( ) Não
( ) Não Lembro

- **Anemia?**

( ) Sim
( ) Não
( ) Não Lembro

- **Varicela (catapora)**

( ) Sim
( ) Não
( ) Não Lembro

- **Outros problemas ?**

( ) Não
( ) Sim, Qual_____

Tente recordar sobre TRÊS PRIMEIROS ANOS DE VIDA seu filho(a) teve alguns desses problemas

- **Episódios múltiplos de febre alta?**

( ) Sim
( ) Não
( ) Não Lembro

- **Infecção no ouvido?**

( ) Sim
( ) Não
( ) Não Lembro

- **Bronquite?**

( ) Sim
( ) Não
( ) Não Lembro

- **Asma?**

( ) Sim
( ) Não
( ) Não Lembro

- **Pneumonia?**

( ) Sim
( ) Não
( ) Não Lembro

- **Infecção de garganta?**

( ) Sim
( ) Não
( ) Não Lembro

- **Uso de Antibiótico?**

( ) Sim
( ) Não
( ) Não Lembro
Se sim, quantas vezes?
( ) 1vez ( ) 3vezes ( ) 5vezes ( ) mais 10 de vezes

Qual antibiótico?_____

- **Uso de outra medicação?**

( ) Sim, Qual?_____
( ) Não
( ) Não Lembro

- **Convulsão?**

( ) Sim
( ) Não
( ) Não Lembro

- **Infecção Urinária?**

( ) Sim
( ) Não
( ) Não Lembro

- **Anemia?**

( ) Sim
( ) Não
( ) Não Lembro

- **Má Nutrição?**

( ) Sim
( ) Não
( ) Não Lembro

- **Outros problemas ?**

( ) Não
( ) Sim, Qual?_____

- **Seu filho é celíaco (intolerante ao glúten)?**

( ) Sim
( ) Não
( ) Não Lembro

- **Seu filho tem alguma intolerância alimentar?**

( ) Sim, Qual? _____
( ) Não
( ) Não Lembro

Muito Obrigada pela sua participação!!

UFPR

Examinador:_____Anotador: _____

Escola:_____Classe:_____Data:_____/___/___

Nome:_____Idade:_____Gênero:_____Raça ( ) Branca ( ) Negra ( ) Asiática ( ) Indígena

	17	16	55 15	54 14	53 13	52 12	51 11	61 21	62 22	63 23	64 24	65 25	26	27
ceo-d / CPO-D														
DDE														
HMI														
HMI														
DDE														
ceo-d / CPO-D														
	46	46	45 85	44 84	43 83	42 82	41 81	31 71	32 72	33 73	34 74	35 75	36	37

c	e	o	ceo-d

C	P	O	CPO-D

ÍNDICE DE CÁRIE		
CPO	*CEO*	
0	A	HÍGIDO
1	B	CARIADO
2	C	REST. + CÁRIE
3	D	REST. SEM CÁRIE
4	E	PERDIDO CÁRIE
5	F	PERDIDO OUTRA RAZÃO
6	G	SELANTE
8	I	Ñ ERUPCIONADO
9	J	EXCLUÍDO
T	T	TRAUMA

HMI			
1. MANCHAS	1 – branca	2- amarela	3-marrom
2. PERDA ESTRUTURA	1- apenas esmalte	2 – esmlate e dentina	
3. RESTAURAÇÃO ATÍPICA	1- satisfatória	2- insatisfatória	
4. EXODONTIA POR HMI			

DDE
0 NORMAL
1 OPACIDADE DEMARCADA
2 OPACIDADE DIFUSA
3 HIPOPLASIA
4 OUTRO
5 OP. DEMARCADA + DIFUSA
6 OP. DEMARCADA + HIPOPLASIA
7 OP. DIFUSA + HIPOPLASIA
8 TODAS AS 3
9 NÃO REGISTRADO

Extensão do DDE	Escore
Normal	0
Menor que 1/3	1
Até 2/3	2
Mais 2/3	3

Má oclusão	
Classe	
Mordida Aberta Ant	
Mordida Cruzada Ant	
Mordida Cruzada Post	
Mordida Profunda	
Sobressaliência Excessiva	
Apinhamento Ant	

Critérios Má Oclusão:
1-Mordida Aberta: Ausência de trespasse vertical cobrindo os incisivos inf
2-Mordida Cruzada Ant: Dois ou mais incisivos superiores ocluindo lingualmente em relação aos incisivos inferiores
3-Mordida Cruzada Post: 2 ou mais molares superiores ocluindo lingualmente em relação aos molares inferiores
4-Mordida Profunda: Trespasse vertical com o incisivo superior cobrindo metade ou mais do compr. Total do incisivo inferior
5-Sobressaliência Excessiva: Distância entre a vestibular do inci. Supe/inf mais que 4mm
6-Apinhamento ant: falta de espaço menor do que a metade do diâmetro medio-distal de um incisivo.

9. ANEXOS

ANEXO 1 – Parecer Consubstanciado do CEP

UNIVERSIDADE FEDERAL DO PARANÁ - SETOR DE CIÊNCIAS DA SAÚDE/ SCS -

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Hipomineralização Molar-Incisivo em uma população sul-brasileira: Prevalência, fatores sistêmicos associados e polimorfismos genéticos

Pesquisador: Juliana Feltrin de Souza Caparroz

Área Temática: Genética Humana:
(Trata-se de pesquisa envolvendo Genética Humana que não necessita de análise ética por parte da CONEP;);

Versão: 3

CAAE: 56829516.0.0000.0102

Instituição Proponente: Departamento de Estomatologia

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 1.689.362

Apresentação do Projeto:

Trata-se de estudo que visa estudar a presença de hipomineralização molar-incisivo em uma população de 960 crianças matriculadas em escolas da rede municipal de ensino de Curitiba. O estudo tem como pesquisadora a Professora Juliana Feltrin de Souza Caparroz e uma equipe de pesquisa composta por Magdalena Raquel Torres Reyes, João Armando Brancher,
Luciana Reichert da Silva Assunção,Lupe Furtado Alle. O estudo será realizado com crianças de oito anos de idade, nas escolas municipais de Curitiba. ALém da coleta de amostras da mucosa bucal para estudo de polimorfismos genéticos e exame bucal para avaliação das condições de saúde bucal das crianças, também será utilizado um questionário a ser aplicado aos pais das crianças a fim de obter informações pertinentes à pesquisa em tela. O estudo terá a duração de 30 meses a partir da aprovação pelo CEP/SD.

Objetivo da Pesquisa:

Objetivo Geral: avaliar a prevalência da Hipomineralização Molar-Incisivo e de hipomineralização em dentes decíduos na população de Curitiba –PR, bem como avaliar a associação dessas condições com cárie dentária, fatores etiológicos sistêmicos, polimorfismos genéticos e o impacto na qualidade de vida das crianças portadoras. Além disto a pesquisadora responsável informa que

Endereço: Rua Padre Camargo, 285 - Térreo
Bairro: Alto da Glória **CEP:** 80.060-240
UF: PR **Município:** CURITIBA
Telefone: (41)3360-7259 **E-mail:** cometica.saude@ufpr.br

UNIVERSIDADE FEDERAL DO PARANÁ - SETOR DE CIÊNCIAS DA SAÚDE/ SCS -

Outros	Modelo2_encaminhamento_ATA.pdf	17:05:15	Souza Caparroz	Aceito
Folha de Rosto	folhaDeRosto.pdf	29/04/2016 16:09:21	Juliana Feltrin de Souza Caparroz	Aceito
Declaração de Pesquisadores	Carta_encaminhamento_projetoCEP.pdf	28/04/2016 17:09:57	Juliana Feltrin de Souza Caparroz	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

CURITIBA, 22 de Agosto de 2016

Assinado por:
IDA CRISTINA GUBERT
(Coordenador)

Endereço: Rua Padre Camargo, 285 - Térreo
Bairro: Alto da Glória **CEP:** 80.060-240
UF: PR **Município:** CURITIBA
Telefone: (41)3360-7259 **E-mail:** cometica.saude@ufpr.br

ANEXO 2 – Autorização Secretaria de Educação

CURITIBA

EDUCAÇÃO

Prefeitura Municipal de Curitiba
Secretaria Municipal da Educação
Superintendência de Gestão Educacional
Departamento de Ensino Fundamental
Av. João Gualberto, 623 7º Andar Torre A
Alto da Glória
80030-000 Curitiba PR
Tel 41 33503076
Fax 41 3350 3047
www.curitiba.pr.gov.br

Curitiba, 05 de abril de 2017.

AUTORIZAÇÃO

Informamos que as pesquisadoras **Aluhe Lopes, Magdalena Torres, Bruna Menoncin e Paula Deschi**, alunas de Mestrado em Odontologia da UFPR, orientadas pela Professora Drª. Juliana Feltrin de Souza, estão autorizadas a realizar pesquisa sobre "Hipomineralização Molar-Incisivo em uma população sul-brasileira: Prevalência, fatores sistêmicos associados e polimorfismos genéticos".

O objetivo é avaliar a prevalência da HMI e hipomineralização em dentes decíduos na população de Curitiba,PR, bem como avaliar a associação dessas condições com cárie dentária, fatores etiológicos sistêmicos, polimorfismos genéticos e o impacto na qualidade de vida das crianças portadoras. Ainda pretende-se investigar se os componentes e propriedades salivares estão relacionados coma severidade da HMI.

As pesquisadoras pretendem:

- Avaliar a prevalência da HMI e hipomineralização em dentes decíduos na população de Curitiba,PR, bem como avaliar a associação desses defeitos de esmalte com a cárie dentária;
- Avaliar o impacto da hipomineralização molar-incisivo na qualidade de vida das crianças;
- Avaliar os fatores sistêmicos associados a HMI nessa população;
- Avaliar a presença de polimorfismosgenéticos relacionados às proteínas de interesse em crianças com HMI e controle;
- Relaciona componentes salivares como cálcio, fosfato, ferro, magnésio e flúor, e propriedades salivares com a severidade da HMI.

Os instrumentos de pesquisa serão a coleta de material biológico para análise bioquímica salivar, questionários aplicados aos pais e entrevista com as crianças para análise de qualidade de vida.

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EDUCAÇÃO

Prefeitura Municipal de Curitiba
Secretaria Municipal da Educação
Superintendência de Gestão Educacional
Departamento de Ensino Fundamental
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Alto da Glória
80030-000 Curitiba PR
Tel 41 33503076
Fax 41 3350 3047
www.curitiba.pr.gov.br

As escolas sorteadas para a pesquisa serão:

SANTA FELICIDADE:

EM Nympha – 3 turmas

EM Pedro Dallabona

EM João Stival

PORTÃO

EM Nova Esperança – 3 turmas

EM São Luiz

EM Itacellina Bittencourt

CIC

EM Álvaro Borges – 3 turmas

EM Pro Morar Barigui – 3 turmas

EM Dom Bosco

BOA VISTA

EM Jaguariaiva – 4 turmas

EM Doutel de Andrade – 3 turmas

EM Anisio Teixeira – 1 turma

PINHEIRINHO

EM Maria Lenkot – 4 turmas

EM José Lamartini – 3 turmas

EM Umuarama – 1 turma

BOQUEIRÃO

EM Germano Pacionik – 3 turmas

EM Rolandia – 2 turmas

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EDUCAÇÃO

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Fax 41 3350 3047
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EM David Carneiro – 1 turma

BAIRRO NOVO

EM Pedro Viriato – 3 turmas

EM Bairro Novo do CAIC/ GLBS – 3 turmas

EM Paulo Freire – 1 turma

CAJURU

EM Prof Maria Marli – 2 turmas

EM Guilherme Braga – 3 turmas

EM Eneas Marques – 2 turmas

EM Linneu F do Amaral – 1 turma

MATRIZ

EM Batel – 2 turmas

EM Vila Torres II – 1 turma

EM Caramuru – 1 turma

A pesquisa será realizada entre os meses de janeiro de 2017 e dezembro de 2018.

Informamos ainda que a decisão final de participar da referida pesquisa caberá aos estudantes e profissionais envolvidos.

Ressaltamos também que o pesquisador deverá entregar **uma cópia impressa e encadernada dos resultados da investigação** para a escola e outra para o Departamento de Ensino Fundamental – Gerência Pedagógica.

Atenciosamente,

Simone Zampier da Silva
Diretora
Departamento de Ensino Fundamental

ANEXO 3 – Registro da revisão sistemática no prospero

UNIVERSITY of York
Centre for Reviews and Dissemination

NHS
National Institute for Health Research

PROSPERO International prospective register of systematic reviews

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What are the systemic factors associated with molar incisor hypomineralization in children and adolescents?

Juliana Feltrin de Souza, Leticia Wambier, Alessandra Reis, Luciana Reichert da Silva Assunção, Fabiano Jeremias, Ana Claudia Chibinski

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Citation

Juliana Feltrin de Souza, Leticia Wambier, Alessandra Reis, Luciana Reichert da Silva Assunção, Fabiano Jeremias, Ana Claudia Chibinski. What are the systemic factors associated with molar incisor hypomineralization in children and adolescents?. PROSPERO 2016:CRD42016035481 Available from http://www.crd.york.ac.uk/PROSPERO_REBRANDING/display_record.asp?ID=CRD42016035481

Review question(s)

What are the systemic factors during the prenatal period associated with molar incisor hypomineralization?

What are the systemic factors occurring during the first three years of child's life associated with molar incisor hypomineralization?

Searches

To identify observation studies to be included for this review, we will search on the electronic databases MEDLINE via PubMed, Scopus, Web of Science, Latin American and Caribbean Health Sciences Literature database (LILACS), Brazilian Library in Dentistry (BBO) and Cochrane Library. We will hand-search the reference lists of all primary studies for additional relevant publications and the related articles link of each primary study in the PubMed database without restrictions to publication date or languages.

No restrictions will be placed on the publication date or languages, and all relevant studies will be translated and reviewed. We will search the abstracts of the annual conference of the International Association for Dental Research (IADR) and their regional divisions (1990–2015) and will get in touch with authors of relevant abstracts for further information. We will explore the grey literature using the database System for Information on Grey literature in Europe (SIGLE), and dissertations and theses using the ProQuest Dissertations and Theses Fulltext database, as well as the Periódicos Capes Theses database. Full text versions of the papers that appear to meet the inclusion criteria will be retrieved for further assessment and data extraction.

Types of study to be included

observational studies

retrospective observational studies

case-control studies

cross-sectional studies

cohort studies

Clinical trial, editorial letters, pilot studies, case reports, historical reviews, in vitro studies, experimental in animals and case series will be excluded.

Condition or domain being studied

Molar incisor hypomineralization

Participants/ population

Inclusion: children aged 6-12 years-old, with the permanent first molars and incisor erupted.

ANEXO 4- Critério de Diagnóstico HMI

Critério para Hipomineralização Molar-Incisivo segundo recomendações da Academia Européia de Odontopediatria. (EAPD)

Escore	Critérios
1. Opacidade demarcada	Defeito demarcado envolvendo alteração na translucidez do esmalte, de graus variados. O esmalte defeituoso tem espessura normal, com superfície lisa, podendo ser branco, amarelo ou marrom.
2. Fratura pós-irruptiva	Defeito que indica perda do esmalte formado após a irrupção dentária. A perda possui bordas irregulares e cortantes, sempre associada a uma opacidade demarcada prévia.
3. Restauração atípica	O tamanho e a forma da restauração não são correspondentes a um preparo para remoção da carie. Em muitos casos, molares tem suas restaurações estendidas para a face vestibular ou palatina/lingual. Frequentemente, as bordas das restaurações apresentam opacidade. Nos incisivos, uma restauração na face palatina pode estar presente, não sendo associada a carie.
4. Exodontia por HMI	Suspeita-se de exodontia por HMI quando: opacidades ou restaurações atípicas em outros primeiros molares permanentes, combinado com a ausência de um primeiro molar. Ausência dos primeiros molares em uma dentição saudável em combinação com opacidades demarcadas em incisivos. Não e comum encontrar incisivos extraídos pela HMI.
5. Não irrompido	Primeiro molar permanente ou o incisivo a ser examinado não irrompido.

ANEXO 5 – Critério para diagnóstico DDE

Critério para classificação dos defeitos de desenvolvimento de esmalte (DDE) segundo FDI, 1992.

Escore	**Características clínicas**
0.Normal	Esmalte com espessura normal e sem alterações de coloração
1.Opacidade demarcada	Esmalte de espessura normal e com superfície intacta, existe uma alteração na translucidez do esmalte, de grau variável. Ela é demarcada a partir do esmalte normal adjacente com limites nítidos e claros, e pode ter a coloração branca, bege, amarela ou marrom.
2.Opacidade difusa	Também uma anormalidade envolvendo uma alteração na translucidez do esmalte, de grau variável, e de coloração branca. Não existe um limite claro entre o esmalte normal adjacente e a opacidade. Pode ser linear ou em placas ou ter uma distribuição confluente.
3.Hipoplasia	Um defeito envolvendo a superfície de esmalte e associado com uma redução localizada na espessura do esmalte. Pode ocorrer de forma de (a) fóssulas únicas ou múltiplas, rasas ou profundas, difusas ou alinhadas, disposta horizontalmente na superfície do dente; (b) sulcos únicos ou múltiplos, estreitos ou amplos (máximo de 2 mm); (c) ausência parcial ou total de esmalte sobre uma área considerável de dentina. O esmalte afetado pode ser translúcido ou opaco.
4.Outros defeitos	
5.Opacidade demarcada e difusa	
6.Opacidade demarcada e hipoplasia	
7.Opacidade difusa e hipoplasia	
8.Todas as três condições juntas	
9. Não registrado	

ANEXO 6 – Critério para risco de viés do artigos incluídos na Revisão Sistemática

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE
COHORT STUDIES

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories. A maximum of two stars can be given for Comparability

Selection

1) Representativeness of the exposed cohort
   a) truly representative of the average _____ (describe) in the community*
   b) somewhat representative of the average _____ in the community *
   c) selected group of users eg nurses, volunteers
   d) no description of the derivation of the cohort
2) Selection of the non exposed cohort
   a) drawn from the same community as the exposed cohort*
   b) drawn from a different source
   c) no description of the derivation of the non exposed cohort
3) Ascertainment of exposure
   a) secure record (e.g. surgical records)*
   b) structured interview*
   c) written self report
   d) no description
4) Demonstration that outcome of interest was not present at start of study
   a) yes
   b) no

Comparability

1) Comparability of cohorts on the basis of the design or analysis
   a) study controls for _____ (select the most important factor) *
   b) study controls for any additional factor* (This criteria could be modified to indicate specific control for a second important factor.)

Outcome

1) Assessment of outcome
   a) independent blind assessment*
   b) record linkage*
   c) self report
   d) no description
2) Was follow-up long enough for outcomes to occur
   a) yes (select an adequate follow up period for outcome of interest) *
   b) no
3) Adequacy of follow up of cohorts
   a) complete follow up - all subjects accounted for*
   b) subjects lost to follow up unlikely to introduce bias - small number lost - > ____ % (select an adequate %) follow up, or description provided of those lost) *
   c) follow up rate < ____% (select an adequate %) and no description of those lost
   d) no statement

Wells, G. A, Shea, B., O'Connel, D. et al. The Newcastle-Ottawa scale (NOS) for assessing the quailty of nonrandomised studies in meta-analyses. http://www ohri ca/programs/clinical_epidemiology/oxford htm 2009 Feb 1

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE
CASE-CONTROL STUDIES

Note: A study can be awarded a maximum of one star (*) for each numbered item within the Selection and Exposure categories. A maximum of two stars can be given for Comparability.

Selection

1) Is the case definition adequate?
   a) yes, with independent validation *
   b) yes, e.g., record linkage or based on self reports
   c) no description
2) Representativeness of the cases
   a) consecutive or obviously representative series of cases *
   b) potential for selection biases or not stated
3) Selection of Controls
   a) community controls *
   b) hospital controls
   c) no description
4) Definition of Controls
   a) no history of disease (endpoint) *
   b) no description of source

Comparability

1) Comparability of cases and controls on the basis of the design or analysis
   a) study controls for _____ (Select the most important factor.) *
   b) study controls for any additional factor * (This criteria could be modified to indicate specific control for a second important factor.)

Exposure

1) Ascertainment of exposure
   a) secure record (eg surgical records) *
   b) structured interview where blind to case/control status *
   c) interview not blinded to case/control status
   d) written self report or medical record only
   e) no description
2) Same method of ascertainment for cases and controls
   a) yes *
   b) no
3) Non-Response rate
   a) same rate for both groups *
   b) non respondents described
   c) rate different and no designation