PREVALENCE, CLINICAL PRESENTATION, AND ASSOCIATED SOCIODEMOGRAPHIC CHARACTERISTICS OF MOLAR HYPOMINERALIZATION IN INDIANA, USA

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Submitted to the faculty of the University Graduate School
in partial fulfillment of the requirements
for the degree
Doctor of Philosophy
in the School of Dentistry,
Indiana University

May 2020

Accepted by the Graduate Faculty of Indiana University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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DEDICATION

To my late father Tagelsir, my mother Zainab, and my twin sister Aida.

To my wonderful daughters; Zainab and Maryam.

To my long-standing friend; my husband Ahmed

And,

To all the kids with MIH in the USA and around the globe, including one of my own!

ACKNOWLEDGEMENT

I am indebted to my family, the ones who are thousands of miles away but very close to my heart, and those who are here: Abdulla's family and Abdulrahman's family who took good care of me and my kids with unconditional love and support.

I am heartfully and genuinely thankful to my mentor; Dr. Martinez, for not only being a research advisor, but a true mentor who guided my steps, gave thoughtful advice for work and life, and handled emotional breakdowns as a sister!

My endless professional supporters, Dr. Gregory, Dr. Soto, and Dr. Dean have been genuine supporters during different stages of the PhD journey, not only in times of joy and celebration of successes, but also during times of struggles and failures.

My special gratitude to my committee members Dr. Lippert, Dr. Ghanim and Dr. Miller, for providing advice and constructive criticism throughout the production of this dissertation.

I am also heartfully grateful to my close friends who were sincere shoulders to lean on; Rawan, Parand, Gina, and Maja, my senior PhD collogues: Grace, Hadeel, Amna, and Dawn, and the whole Oral Health Research Institute family especially Dr. Gossweiler, Cynthia and Melissa.

I am especially grateful to the IUSD SEAL Indiana faculty, staff and students who helped during data collection and were definitely behind the accomplishment of the second and third projects. Additionally, special thanks are due to all Indiana public schools' staff, nurses, teachers, students, and their parents who were part of these projects.

Lastly, I would like to extend my appreciation to my former coworkers and students at the University of Khartoum, Sudan.

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Molar Hypomineralization (MH) of the first permanent molars (FPMs) and the second primary molars (SPMs) is a common developmental defect of enamel, with global prevalence of 14% and 5% respectively. Children with MH represent a special pediatric population because their affected molars have extreme susceptibility to enamel breakdown, decay and tooth sensitivity. Although the problem of MH has been described almost twenty years ago mainly through reports from Europe, there is very little information about the problem from the USA. In this dissertation, MH was explored both from the perspectives of pediatric dentists' (PDs) and at population level. The majority of the survey respondents perceived MH prevalence to be <10% in their clinical practice (62%). The most cited clinical challenge in managing MH teeth was "long-term success of restorations" (79%). When analyzed individually, responses differed significantly for different demographics and educational characteristics of the respondents ($p<0.05$). At population level, MH of the FPMs (Molar Incisor Hypomineralization (MIH) cohort: 337 schoolchildren, average age 9 years) and of the SPMs (Hypomineralized Second Primary Molar (HSPM) cohort: 423 schoolchildren, average age 7 years) had prevalence estimates of 13% and 6% respectively. In the MIH cohort, water fluoridation or non-Hispanic Black race/ethnicity was significantly associated with higher collective prevalence of enamel defect (EDs) ($P<0.05$), but not with the prevalence of MH of the FPMs. In the HSPM cohort, race/ethnicity was significantly associated with higher overall prevalence

of EDs of SPMs, but not with the HSPM prevalence. Older age group (>10 years), living in central Indiana, and water fluoridation were significantly associated with higher overall prevalence of EDs ($P<0.01$), but not with the HSPM prevalence. Caries experience was significantly higher in children with MH of FPMs and/or SPMs than in the group without MH. We concluded that USA pediatric dentists' respondents were well aware of the MH problem, but demonstrated discrepancies in different aspects of the MH problem. At population level, MIH and HSPM were common presentation with prevalence estimates similar to the global figures. Certain demographic characteristics were significantly associated with the overall prevalence of the enamel defects of the examined teeth.

E. Angeles Martinez Mier, DDS PhD, MSD, Chair

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LIST OF ABBREVIATIONS

AED: All observed enamel defects, including MIH

DDE: Developmental Defect of Enamel

DMFS: permanent decayed, missing (due to caries) and filled surfaces

dmfs: primary decayed, missing (due to caries) and filled teeth

DMFT: permanent decayed, missing (due to caries) and filled teeth

dmft: primary decayed, missing (due to caries) and filled teeth

DO: Diffuse opacities

EAPD: European Academy of Pediatric Dentistry

ED: Enamel Defect

FPM: First Permanent Molar

HSPM: Hypomineralized Second Primary Molar

ICDAS: International Caries Detection and Assessment System

mDDE: modified Developmental Defect of Enamel

MIH: Molar Incisor Hypomineralization

MH: Molar Hypomineralization

PEB: Post-Eruptive Breakdown

PI: Permanent Incisor

PPM: Part Per Million

SPM: Second Primary Molar

U.S.: United States

USA: United States of America

CHAPTER 1: GENERAL INTRODUCTION

Molar incisor hypomineralization (MIH) is defined as a spectrum of qualitative demarcated developmental enamel opacities affecting the first permanent molars with or without involvement of the permanent incisors (Weerheijm, Jalevik et al. 2001, Weerheijm, Duggal et al. 2003). In the primary dentition, comparable hypomineralization defects affecting the second primary molars have been described as well. The terms "Deciduous Molar Hypomineralization (DMH)" (Elfrink, Schuller et al. 2008) and "Hypomineralized Second Primary Molars (HSPM)" (Ghanim, Manton et al. 2013) have been used concomitantly describing the same hypomineralization defects that affects one to four second primary molars. These defects are distributed in an asymmetrical fashion and have discernible variations in severity ranging from small white, yellow, or brown opacities to severe defects with post-eruption disintegration of enamel (Weerheijm, Jalevik et al. 2001, Weerheijm 2003, Weerheijm, Duggal et al. 2003). These opacities are very distinct from the diffuse opacities caused by fluorosis in that they have well defined and discrete borders from adjacent normal enamel (Weerheijm 2003).

1.1. Background on Enamel Maturation

The process of enamel formation -referred to as amelogenesis- is essentially initiated as the secretory-stage ameloblasts lay down partially mineralized enamel matrix forming the entire thickness of enamel (Smith 1998). The organic enamel matrix, -which is highly heterogeneous, and fundamentally made of Amelogenins (~90%)-regulates mineralization and growth of enamel crystallites (Robinson, Brookes et al. 1998, Smith 1998). In the subsequent transition and maturation stages of amelogenesis, a series of morphological and cellular modulations of ameloblasts take place resulting in the

degradation of enamel matrix proteins and extensive deposition of minerals (Smith 1998). Removal of enamel matrix proteins by extracellular proteinases (matrix metalloproteinase-20 and kallikrein-4) -secreted by ameloblasts at different stages of amelogensis (Lu, Papagerakis et al. 2008, Simmer, Hu et al. 2009) - is a prerequisite for fluid and ions movements into the enamel matrix, extensive minerals uptake, and volumetric growth of crystals both in width and thickness (Robinson 2014). Enamel formation is a very slow process in which as much as ~65% of development time is devoted to the maturation stage of amelogenesis (Smith 1998). Moreover, the span of enamel maturation process is also extremely variable both between different species and teeth types, for example the maximum mineralization of a rat incisor may take 10-14 days while the first sign of mineralization of the first permanent human molar manifests around or soon after birth but extends up to several years thereafter (Robinson 2014). By the termination of enamel formation, mature enamel is composed of approximately 95% mineral and less than ~2% by weight organic material (Smith 1998). Insults ensuing during the later stages of amelogenesis produce enamel of normal thickness at the time of tooth eruption, yet with soft porous hypomineralized quality (Suckling 1989).

1.2. Histomorphology of MIH Enamel and Associated Dental Tissues

Enamel

At a histo-morphological level, the properties of MIH-affected enamel are highly variable, both within and between lesions in terms of reduced mineral content, reduced hardness, increased porosity, and increased carbonate (Crombie, Manton et al. 2013) and protein content (Farah, Monk et al. 2010). Qualitative descriptions of demarcated enamel defects have shown that these lesions follow the typical distribution of the incremental

lines of Ritzus (Jalevik and Noren 2000, Gambetta-Tessini, Marino et al. 2017). The studies also confirmed that the ameloblasts of hypomineralized enamel are capable of forming the entire thickness of enamel validating the notion that the contributory insults take place after the secretory stage of amelogenesis (Fagrell, Salmon et al. 2013, Gambetta-Tessini, Marino et al. 2017) and differentiating the defect from hypoplasia where the putative insults dominate during the secretory stages of amelogenesis resulting in pathologically thin and deficient enamel (Alaluusua 2010).

X-ray microtomography investigation of hypomineralized enamel of teeth with MIH showed that the mineral density (MD) of hypomineralized enamel is almost one fifth that of sound enamel. Unlike sound enamel, the mineral density values of MIH affected enamel decrease from the DEJ to the occlusal region, then increase again at the cusp tip with the highest mineral density values found midway between the DEJ and the outer enamel (Farah, Swain et al. 2010). Studies using micro-computed tomography (micro-CT) of MIH- and HSPM-affected enamel confirmed the matching gradient decrease of MD from DEJ to outer enamel contrasting that of sound enamel (Elfrink, ten Cate et al. 2013, Gambetta-Tessini, Marino et al. 2017).

Polarized light microscopy (Jalevik and Noren 2000, Crombie, Manton et al. 2013) as well as Micro-CT (Gambetta-Tessini, Marino et al. 2017) investigations have disclosed that MIH-affected enamel appears severely porous; however, the surface enamel layer demonstrated an increased hardness and mineral content and a decreased porosity relative to the underlying subsurface lesion presumably related to post-eruptive remineralization related to fluorides and mineral exchange from the oral environment (Elfrink, ten Cate et al. 2013).

The varying clinical appearance and the degree of staining of demarcated opacities reflects their degree of subsurface porosity and MD values, which are correlated with their liability to surface disintegration after eruption. The yellow-brown defects appear to extend through the whole enamel layer and are more porous and more prone to post-eruptive breakdown than the white-cream opacities which are situated in the inner parts of the enamel (Jalevik, Dietz et al. 2005, Farah, Drummond et al. 2010, Gambetta-Tessini, Marino et al. 2017).

Scanning electron microscopy (SEM) studies investigating the ultrastructure of hypomineralized enamel of FPMs, on the other hand, have shown that the basic enamel structure, with hydroxyapatite crystals forming rods and interrod enamel, is preserved in the porous zones as well as in the well-mineralized zones of the enamel. The packing of the crystals, however, was described to be amorphous, loose, and less tight in the porous parts but with a well-defined border between the normal and porous areas running parallel to the rods (Jalevik, Dietz et al. 2005). Further, the altered prism sheath structure extends from the affected opaque enamel to the apparent translucent enamel of the transition region adjacent to the opaque enamel with an ensuing significant decline in the flexural strength not only of the MIH affected enamel but also of the immediate transition enamel adjacent to the sound enamel (Chan, Ngan et al. 2011).

Dentin

Regarding the structure of dentin, scanning electron microscopy (SEM) and polarized light microscopy (PLM) reports failed to show any structural changes of the dentin underlying hypomineralized enamel (Heijs, Dietz et al. 2007), yet a recent study utilizing 3D Micro-CT analysis was able to reveal reduced mineral density in dentin

especially below the severe spectrum of MIH lesions (post-eruptive breakdown lesions) (Gambetta-Tessini, Marino et al. 2017).

Dental pulp

Alterations in the dental pulp of MIH teeth may be directly correlated with the apparent thermal hypersensitivity and failure to achieve adequate local anesthesia in these teeth. However, very limited enquiry into the underlying pathophysiology of dental pulps of MIH teeth is accessible in the dental literature. A pioneer histochemical analysis of teeth affected with MIH revealed that the pulp vascularity makeup was not significantly different from pulps of sound teeth. However, there was an anomalous pulpal innervation density and increase accumulation of immune cells in the pulpal tissues of teeth affected with MIH when compared to sound teeth (Rodd, Boissonade et al. 2007).

Rod et al, 2007 conducted a quantitative immune-cytochemical analysis. Their results showed pulpal tissues of MIH affected teeth had significantly higher mean neural and vascular expression of the noxious heat receptor known as the "transient receptor potential ion channel" (TRPV1), irrespective of the severity of the hypomineralization defect, (i.e. with or without enamel breakdown) when compared to the pulp tissues of sound non-affected teeth (Rodd, Morgan et al. 2007). Fagrell et al., 2008 showed that in MIH affected teeth, oral bacteria are more likely to penetrate through enamel into the dentine even with apparently intact hypomineralized enamel, resulting in compromised dental pulp status (Fagrell, Lingstrom et al. 2008).

Morphology of HSPM

Reports investigating the histo-morphology of HSPMs are scarce, possibly because of the lower prevalence rate of HSPMs when compared to hypomineralization

defects of FPMs. Analogous to hypomineralized FPMs, micro CT analysis of HSPMs have disclosed that the mineral density in affected primary molars was significantly lower (20-22% less MD) in yellow and brown but not white demarcated opacities when compared to that of sound enamel (Elfrink, ten Cate et al. 2013).

1.3. Clinical Implications of MIH and HSPM

Dental caries in teeth with MIH

There is an established evidence that permanent teeth with demarcated hypomineralization of MIH have higher DMF indices and caries prevalence than teeth without MIH (Kotsanos, Kaklamanos et al. 2005, Muratbegovic, Markovic et al. 2007, Groselj and Jan 2013, Jeremias, de Souza et al. 2013, Petrou, Giraki et al. 2014, Pitiphat, Savisit et al. 2014, Kosma, Kevrekidou et al. 2016, Grossi, Cabral et al. 2017). This has been confirmed in a recent review assessing the correlation between MIH and dental caries in permanent teeth, however, the authors recommended that the conclusions to be interpreted with caution since none of the reviewed studies was considered a high-quality study (Americano, Jacobsen et al. 2017).

Dental caries in teeth with HSPM

Likewise, children with HPSMs have increased likelihoods of having dental caries (Elfrink, Schuller et al. 2008, Oyedele, Folayan et al. 2016), and second primary molars with hypomineralization (HSPMs) were found three times more prone to develop advanced carious lesions (ICDAS codes 4–6) than a defect-free molar (Ghanim, Manton et al. 2013). A current meta-analysis based on population-based studies of developmental defects of enamel (DDE) in primary teeth revealed that children with DDE in general have higher probabilities of having dental caries. The meta-analysis showed that primary

teeth with demarcated opacities, unlike those with hypoplasia and diffused opacities, exhibited no significant higher odds of having dental caries. However, when only studies with molar hypomineralization were included in the analysis, the pooled estimate analysis showed significant association with dental caries (Costa, Silveira et al. 2017).

Enamel disintegration

The compromised physical characteristics of MIH hypomineralized enamel accelerate the risk of tooth structure loss, exposure of dentin, and subsequently the development of dental caries (Weerheijm, Jalevik et al. 2001, Weerheijm 2003, Fragelli, Jeremias et al. 2015). Susceptibility of hypomineralized enamel for physical disintegration have been designated as one of the characteristic clinical features of MIH and could occur as soon as the tooth is under masticatory forces resulting in defects that can be mistaken for enamel hypoplasia (Weerheijm 2003).

Hypersensitivity

Increased sensitivity to thermal and mechanical stimuli is a frequent finding in MIH teeth (Weerheijm, Jalevik et al. 2001, Weerheijm 2003), although the exact mechanism is not well understood. However, assumptions such as the permeation of bacteria through the porous hypomineralized enamel and subsequent subclinical pulp inflammation (Fagrell, Lingstrom et al. 2008) and the anomalous pulpal innervation density in the pulp horn and sub-odontoblastic region of hypomineralized teeth (Rodd, Boissonade et al. 2007) have been suggested.

Despite the very limited available literature exploring the issue of teeth hypersensitivity in MIH, hypersensitivity has been documented not only as a self-reported problem but rather as an objective measure using standardized indices (Ebel,

Bekes et al. 2018, Raposo, de Carvalho Rodrigues et al. 2019) in teeth affected with MIH when compared to control teeth.

Dental treatment needs, behavioral issues, and oral- health related quality of life

With all the aforementioned factors considered, children with MIH- affected teeth have been found to endure greater dental treatment burden including more frequent dental visits (Ghanim, Manton et al. 2012), higher failed restorations rate (Leppaniemi, Lukinmaa et al. 2001), and more repeated dental treatments and considerable behavior management problems and dental fear and anxiety (Jalevik and Klingberg 2002). Furthermore, children with severe MIH defects suffered a greater negative impact on the oral health related quality of life -especially in the oral symptoms and functional limitations domains- when compared to those not affected with MIH (Dantas-Neta, Moura et al. 2016).

1.4. Diagnostic Criteria of MIH and HSPM

The diagnostic criteria most extensively used in the literature for MIH is the modified Developmental Defect of Enamel (mDDE) index proposed by the federation dentaire international in 1992 (Clarkson 1989) and the EAPD criteria suggested by Weerheijm et al. (Weerheijm, Duggal et al. 2003). Briefly, DDEs are classified as demarcated opacities, diffuse opacities, and hypoplasia.

In 2001, three similar studies (Jalevik, Klingberg et al. 2001, Leppaniemi, Lukinmaa et al. 2001, Weerheijm, Groen et al. 2001) were able to point out to the occurrence of demarcated enamel defects of FPMs and formed the cornerstone for the description and nomenclature of the condition of MIH. Subsequently, the EAPD seminar in Athens 2003 established the EAPD judgment criteria for MIH in epidemiological

studies (Table A.1.) as the modified DDE index (mDDE) proved to be too time consuming, not adequate for MIH studies, and excluded post-eruptive breakdown (PEB) which is a prominent feature of MIH (Jalevik 2010). Although the established EAPD criteria have been used widely since their description in 2003, prevalence rates of MIH reported in epidemiological studies have shown great discrepancies. For these reasons, Weerheijm 2015 proposed the need for a worldwide-accepted MIH standardized scoring and calibration system (Weerheijm 2015). Recently, Ghanim et al.,2015 (Ghanim, Elfrink et al. 2015) proposed a unified, practical charting method (Table A.2.) that integrates both the elements of the EAPD criteria and the modified index of developmental defects of enamel (mDDE index) for grading the clinical status of MIH and its extent on the involved tooth surface. This new assessment tool has reasonable validity and reliability to be used in clinical screenings and population-based studies (Ghanim, Marino et al. 2019).

1.5. Etiology and Risk Determinants of MIH and HSPM

Systematic and environmental factors-MIH

In general, systemic insults that disturb ameloblasts during the secretory stage result in pathologically thin, hypoplastic enamel while those occurring during the transitional and maturation stages of amelogenesis result in hypomineralized, hypomatured enamel of normal thickness (Alaluusua 2010). The first permanent molars start to develop during the fourth month of gestation, show the first sign of mineralization in cusp tips around or soon after birth, and commence early maturation phase during the first year of life (Logan and Kronfeld 1933). However, enamel maturation of the FPMs takes several years (late maturation stage) and systemic disturbances after the first year may be associated with MIH (Alaluusua 2010). Therefore, environmental, systemic,

medical, and genetics factors that disturb maturation procedure during pregnancy and the first three years of life have been linked to MIH (Crombie, Manton et al. 2009, Alaluusua 2010, Fagrell, Ludvigsson et al. 2011, Fatturi, Wambier et al. 2019).

These potential systemic etiological factors have been identified in three different periods during development; pre-, peri- and postnatal periods. In an attempt to identify these putative etiological factors in children with MIH, several retrospective, case-control, and prospective studies (Silva, Scurrah et al. 2016, Fatturi, Wambier et al. 2019) have been published.

Prenatal medical condition and risk factors: Factors as maternal illnesses, maternal psychological stress, smoking, consumption of alcohol, and maternal medications have been investigated in correlation with MIH. MIH was significantly more common among those whose mothers had experienced problems during pregnancy compared to controls (Lygidakis, Dimou et al. 2008, Crombie, Manton et al. 2009). Of all the prenatal risk factors investigated, a recent meta-analysis (Fatturi, Wambier et al. 2019) has associated maternal illnesses and psychological distress during pregnancy with higher odds of MIH.

Perinatal medical conditions: controversial results exist regarding the effect of disturbance during this period. Caesarian section (Lygidakis, Dimou et al. 2008, Pitiphat, Savisit et al. 2014), prolonged delivery and premature birth (Lygidakis, Dimou et al. 2008) were common perinatal conditions in MIH affected subjects compared to controls. However, other similar studies were not able to confirm this correlation (Dietrich, Sperling et al. 2003, Crombie, Manton et al. 2009). On the other hand, hypoxia related to birth complications such prematurity and respiratory stress have been suggested as a risk

factor of MIH (Lygidakis, Dimou et al. 2008). In a recent meta-analysis (Fatturi, Wambier et al. 2019), only cesarean section and delivery complications of the perinatal conditions were associated with higher odds of MIH.

Postnatal medical conditions: Direct correlation between postnatal medical problems and MIH has been suggested in numerous studies (Beentjes, Weerheijm et al. 2002, Lygidakis, Dimou et al. 2008, Ghanim, Manton et al. 2013, Wuollet, Laisi et al. 2016). Numerous reports suggested that high fever (Jalevik, Noren et al. 2001, Beentjes, Weerheijm et al. 2002, Lygidakis, Dimou et al. 2008, Elfrink, Moll et al. 2014), bronchitis, asthma and respiratory illnesses in the first four years (Jalevik, Noren et al. 2001, Pitiphat, Luangchaichaweng et al. 2014, Tourino, Correa-Faria et al. 2016), otitis media (Beentjes, Weerheijm et al. 2002, Wuollet, Laisi et al. 2016), and chickenpox (Chawla, Messer et al. 2008, Whatling and Fearne 2008) were all significantly associated with MIH. A meta-analysis published recently has only associated respiratory illnesses (asthma, bronchitis, rhinitis, and other breathing problems) and high fever of all other medical problems in the postnatal period with higher odds of MIH (Fatturi, Wambier et al. 2019).

The use of antibiotics in general during the early postnatal period has been linked with MIH in many studies (Jalevik, Noren et al. 2001, Beentjes, Weerheijm et al. 2002, Wuollet, Laisi et al. 2016). The use of amoxicillin (Whatling and Fearne 2008, Laisi, Ess et al. 2009, Wuollet, Laisi et al. 2016) and penicillin or macrolides (Wuollet, Laisi et al. 2016) during the first year of life has also been implicated with MIH. Although an earlier classic Swedish study contradicted these findings (Koch, Hallonsten et al. 1987), animal-based X-ray microtomography study in piglets suggested a reduction in mineral density

of FPMs when average and high doses of amoxicillin were administered (Kuscu, Sandalli et al. 2013).

Exposure to environmental toxicants: the involvement of endocrine disturbing chemicals (polychlorinated biophenyls (PCBs), dioxins, and Bisphenol A (BPA) as risk determinants associated with MIH has been proposed in many studies (Alaluusua, Lukinmaa et al. 1996, Laisi, Kiviranta et al. 2008, Jedeon, De la Dure-Molla et al. 2013, Jedeon, Marciano et al. 2014). Nevertheless, these associations remain controversial (Laisi, Kiviranta et al. 2008, Kuscu, Caglar et al. 2009).

Genetics-MIH

Comprehensive, large cohort studies investigating possible genetic associations with MIH do not exist in the current literature (Kuhnisch, Thiering et al. 2015, Jeremias, Pierri et al. 2016). The SCUBE 1 gene was recognized as a potential genetic locus for MIH in a genome-wide association study with a total of 2,013,491 single-nucleotide polymorphisms (SNPs) analyzed. However, the study was underpowered and lacked independent sample replication (Kuhnisch, Thiering et al. 2015). A recent family-based genetic study demonstrated that genetic variations in the AMELX, BMP4, FGFR1 and other genes were associated with MIH (Jeremias, Pierri et al. 2016). Supporting the multifactorial genetic contribution to MIH, a recent twin study has demonstrated a significant higher concordance rates in mono- versus dizygotic twins (Teixeira, Andrade et al. 2018).

Other Risk Determinants-MIH

Race and Ethnicity: On the other hand, there is little evidence in the literature correlating ethnicity and health-related inequality with MIH. Dutch ethnicity and Malay

ethnicity were identified as risk factors for HSPM and MIH respectively (Elfrink, Moll et al. 2014, Ng, Eu et al. 2015). However, most studies were not performed in a large multi-ethnic cohort; therefore, the influence of ethnicity has not been well explored in the literature regarding the prevalence of MIH.

Gender: the gender influence on MIH prevalence has been a conflicting issue in many studies (Chawla, Messer et al. 2008, Zawaideh, Al-Jundi et al. 2011, Garcia-Margarit, Catala-Pizarro et al. 2014). However, an analysis of seventy MIH prevalence studies revealed no gender predilection effect on MIH prevalence estimates (male 14.3%, 95% CI: 12.0–16.6, and female14.4%, 95% CI: 12.8–15.9) (Zhao, Dong et al. 2018).

Socioeconomic factors: exploring the MIH risk factors within the context of socioeconomic status have been mostly disputable. Several studies have failed to find significant association between MIH and socioeconomic indicators such as household annual income and parents' education (Casanova-Rosado, Medina-Solis et al. 2011, Jeremias, de Souza et al. 2013, Tourino, Correa-Faria et al. 2016). However, two studies from Brazil and Argentina have reported significantly higher odds of having MIH in children of families with higher annual income (Biondi, Cortese et al. 2011, Teixeira, Andrade et al. 2018).

Systematic and other factors-HSPM

The available evidence on the etiology of HSPM is as mutually as weak as that of MIH, with definite lack of conclusive cause –effect relationship. Few studies have explored risk factors associated with the demarcated hypomineralization defects of the second primary molars (Elfrink, Moll et al. 2014, Silva, Kilpatrick et al. 2019). A population-based prospective cohort study identified certain prenatal (Dutch ethnicity and

alcohol consumption by the mother during pregnancy), perinatal (low birth weight), and postnatal (fever episodes in the first year of life) risk factors as significant determinants of HSPM (Elfrink, Moll et al. 2014).

Unlike the alleged genetic influence on MIH from the single twin study available in the literature (Teixeira, Andrade et al. 2018), similar investigation in twins has revealed that concordance rates of HSPM among monozygotic and dizygotic twins were not significantly different, which challenges the role of genetics in the etiology of HSPM. On the other hand, the same prospective twin study exposed rather a stronger association between environmental factors and HSPM such as vitamin D levels at birth, infantile eczema, in vitro fertilization, socioeconomic status, and maternal smoking beyond the first trimester of pregnancy (Silva, Kilpatrick et al. 2019).

1.6. Epidemiological Data of MIH and HSPM

The earliest MIH prevalence reports were from Scandinavia (Koch, Hallonsten et al. 1987, Alaluusua, Lukinmaa et al. 1996, Alaluusua, Lukinmaa et al. 1996, Jalevik, Klingberg et al. 2001, Leppaniemi, Lukinmaa et al. 2001) and the Netherlands (Weerheijm, Groen et al. 2001). Extensive prevalence data followed from the rest of the European continent, Asia, the Oceania, Africa, and South America. Nevertheless, published prevalence figures for MIH show large variations, between 3% to 44% and 0.5%-40% for MIH (Lygidakis, Dimou et al. 2008, Zhao, Dong et al. 2018). This discrepancy in prevalence data has been attributed to differences in socio-behavioral, environmental, and genetic factors of the studied populations, as well as lack of standardized protocols, study design, and conventional scoring systems (Elfrink, Ghanim et al. 2015).

Europe

The European continent has been the most prolific in publishing MIH prevalence reports with around 45 studies from 17 different countries. The highest prevalence estimates were 40% and 37% from Leeds, UK (Balmer, Laskey et al. 2005) and Denmark (Wogelius, Haubek et al. 2008) respectively. The lowest estimates were 5% and 6% from Bulgaria (Kukleva, Petrova et al. 2008) and Germany (Dietrich, Sperling et al. 2003, Preusser, Ferring et al. 2007) respectively. Studies reporting on the MIH prevalence from Europe had large variations in sample sizes and recruitment settings. Some of these reports (Koch, Hallonsten et al. 1987, Dietrich, Sperling et al. 2003, Kukleva, Petrova et al. 2008, Balmer, Toumba et al. 2012) were based on national epidemiological surveys with sample size larger than 2000 children.

Asia

Asia has the second most published prevalence data on MIH from 13 different countries and around 29 studies. The earliest study was from Hong Kong in 2008 (Cho, Ki et al. 2008) and the latest was from India (Rai, Singh et al. 2018) and Japan (Saitoh, Nakamura et al. 2018). Almost half of the studies were conducted in India, but these studies showed great variations in prevalence estimates than those conducted within the same country. In general, the lowest prevalence was around 3% from Hong Kong (Cho, Ki et al. 2008) and the highest was around 28% from Thailand (Pitiphat, Luangchaichaweng et al. 2014).

The Oceania

Out of the 14 countries and territories, A total of five studies from three cities in Australia and one region in New Zealand were identified from the Oceania. Except for

one study (Balmer, Laskey et al. 2005), all the other prevalence studies were conducted exclusively among schoolchildren and showed limited variations ranging between 22% in Perth (Arrow 2008) to 15% in Wainuiomata children (NZ)(Mahoney and Morrison 2009) and Melbourne children (Gambetta-Tessini, Marino et al. 2018). Pooled analysis estimates place Oceania as the continent with the second highest MIH prevalence of 16.3%, 95% CI: 12.6–20.0 (Zhao, Dong et al. 2018).

South America

There are around 12 different studies exploring the MIH prevalence from the South American countries. Eight of these reports are from different regions of Brazil including the earliest study from South America in 2009 (Soviero, Haubek et al. 2009). The latest studies were from Chile (Gambetta-Tessini, Marino et al. 2019) and Colombia (Mejia, Restrepo et al. 2019). The variations in prevalence estimates was extreme with the highest estimate around 40% (Brazil)(Soviero, Haubek et al. 2009) and the lowest around 6% from two university clinics in Uruguay and Argentina (Biondi, Cortese et al. 2011, Biondi, Lopez Jordi Mdel et al. 2012). Overall, pooled analysis estimates of MIH prevalence showed that South America has the highest MIH estimate (18.0%, 95% CI: 13.8– 22.2) (Zhao, Dong et al. 2018).

Africa

Out of the total 54 African countries, only four countries have published prevalence reports on MIH (Nigeria, Kenya, Libya and Egypt). However, in total there are six studies and half of these studies were conducted in Nigeria with a prevalence estimate ranging between 3%-18%. The earlier prevalence study was from Libya (Fteita, Ali et al. 2006) and had the least MIH prevalence estimate of 3%. The newest report was

from Egypt (Saber, Waly et al. 2018) and had a prevalence estimate similar to the Libyan study (3%). Pooled prevalence estimates based on continent showed that Africa had the least MIH prevalence estimates (10.9%, 95% CI: 4.2–17.6) (Zhao, Dong et al. 2018).

North America

Even with the large number of prevalence studies available worldwide, there is a notable gap in prevalence studies coming from the North American region (Elfrink, Ghanim et al. 2015). It remains unexplained why the USA as one of the largest countries in the world by mass area and by population had not scrutinized the prevalence of MIH. So far, there are two studies from North America. The first is from Mexico City (Gurrusquieta, Nunez et al. 2017) and the other one is a pilot study from Wisconsin state in the Midwest region of the USA (Davenport, Welles et al. 2019). The two studies reported variable prevalence estimates of 16% from Mexico City (Gurrusquieta, Nunez et al. 2017) and 10% from Milwaukee city in Wisconsin (Davenport, Welles et al. 2019).

Epidemiological data-HSPM

Similar to the variations in MIH prevalence estimates, HSPM defect prevalence show similar variations between 5% from the Netherlands, Nigeria, and Chile (Elfrink, Schuller et al. 2008, Temilola, Folayan et al. 2015, Gambetta-Tessini, Marino et al. 2019) to 20% (Silva, Kilpatrick et al. 2019) from Australia. However, the number of available studies is much fewer than the MIH studies, with a total of 14 studies from 10 countries around the globe.

Europe

The earliest study exploring the hypomineralized second primary molar prevalence was from the Netherlands in 2008 (Elfrink, Schuller et al. 2008). This was

followed by another study from the Netherlands in 2012 (Elfrink, ten Cate et al. 2012), Germany in 2014 (Kuhnisch, Heitmuller et al. 2014), and Spain in 2016 (Negre-Barber, Montiel-Company et al. 2016). The prevalence estimates in all the studies were below 10%, except for the Spanish study where the prevalence of HSPMs was as high as 15%.

Asia

There are three studies from the Asian continent examining the prevalence estimates of HSPM, one from Iraq (Ghanim, Manton et al. 2013) and two from India (Mittal and Sharma 2015, Goyal, Dhareula et al. 2019). All three studies implemented the EAPD description as a diagnostic index and reported very similar HSPM estimates between 6% and 8%.

The Oceania

There is a total of three studies exploring the prevalence of HSPMs from the Oceania. All three studies (Gambetta-Tessini, Marino et al. 2018, Owen, Ghanim et al. 2018, Silva, Kilpatrick et al. 2019) were carried out in Melbourne and were published in 2018 and 2019. However, variations in prevalence estimates between 20% (Silva, Kilpatrick et al. 2019) and 8% (Gambetta-Tessini, Marino et al. 2018) were reported.

Africa

Nigeria is the only country in Africa that have reported the prevalence of HSPMs in two separate studies (Temilola, Folayan et al. 2015, Oyedele, Folayan et al. 2016). The studies used different diagnostic criteria but had similar estimates between 5% and 6%.

The Americas

Only two South American countries have reported on HSPMs prevalence. The studies, one from Brazil (da Silva Figueiredo Se, Ribeiro et al. 2017) and the other from

Chile (Gambetta-Tessini, Marino et al. 2019) used the EAPD and the updated EAPD-mDDE diagnostic indices respectively but reported similar prevalence estimates between 5% and 6%. No similar studies employing the EAPD diagnostic criteria or its modified version were reported from any of the North American countries.

1.7. Perception of Molar Incisor Hypomineralization Among Oral Health Professionals

Recognition of MIH as a clinical problem from the perspective of oral health professionals can be considered as a concrete step to explore this problem, especially in regions where the actual estimates of the problem are scarce or non-existing. Surveys of the members of European (Weerheijm and Mejare 2003) and Australian⁄New Zealand (Crombie, Manton et al. 2008) societies of pediatric dentistry, in addition to reports from some Middle Eastern (Ghanim, Morgan et al. 2011, Silva, Alhowaish et al. 2016), Asian (Hussein, Ghanim et al. 2014, Gamboa, Lee et al. 2018), and South American countries (Gambetta-Tessini, Marino et al. 2016) have identified that the majority of oral health professionals perceived MIH to be a serious clinical problem of public health consequences. Most of these reports provided consistent results on the apparent familiarity of oral health care professionals (pediatric dentists, general dental practitioners, nurses, and dental academician) with teeth showing the typical picture of MIH (Weerheijm and Mejare 2003, Crombie, Manton et al. 2008, Ghanim, Morgan et al. 2011, Hussein, Ghanim et al. 2014). These reports also showed remarkable discrepancies regarding the estimated MIH prevalence (Ghanim, Morgan et al. 2011, Silva, Alhowaish et al. 2016). Moreover, in some of these reports, pediatric dentists had significantly higher MIH knowledge scores (Gamboa, Lee et al. 2018) or reported higher perceived

MIH prevalence estimates (Crombie, Manton et al. 2008) than general dental practitioners. Table A.3. illustrates some of the available literature on the topic of MIH perception and experience of oral health professionals.

On the views expressed on the etiology of MIH, consensus among respondents' in most studies was reached that MIH is a multifactorial condition (Crombie, Manton et al. 2008, Ghanim, Morgan et al. 2011). Most Australian and New Zealand pediatric dental clinicians identified medical conditions as the most common etiological factor. Some Middle Eastern dental practitioners agreed on genetic factors (Silva, Alhowaish et al. 2016) while others could not reach apparent agreement on a specific etiological factor (Ghanim, Morgan et al. 2011). Considerable percentages of respondents' from both the Australian/NZ and the Middle Eastern reports agreed that the incidence of MIH was increasing (Crombie, Manton et al. 2008, Ghanim, Morgan et al. 2011). However, the latter statement remains controversial (Elfrink, Ghanim et al. 2015). Surprisingly, data from the United States investigating the oral health professionals and pediatric dentists' level of perception regarding MIH are nonexistent.

The hypothesis of my first study was that oral health professionals' in the Midwest region of the USA have considerable inconsistency in their awareness and understanding of the MIH/HSPM problems. Targeting USA pediatric dentists would be a preliminary step to highlight MIH as an existing dental problem and understand perceptions from the dental professionals' perspective. Data would serve as the baseline for a broader USA survey and pinpoint justifications to further examine the epidemiological prevalence of the MIH problem, not only in the Midwest but also across the different geographical regions of the USA.

For the second and third projects, I hypothesized that at epidemiological level, there is a difference in the prevalence of MIH and HSPM in Indiana when compared to the estimated global averages. To assess theses hypotheses, the following studies were designed and executed as follows:

Specific aim 1 (Chapter 2): The project aimed at investigating the level of perception of the MIH problem among pediatric dentists practicing in the U.S. Midwest region, determining their insight of the problem's frequency in their practice, and their diagnostic, clinical challenges and management strategies of MIH. Pediatric dentists identified by the AAPD's 2016-2017 membership directory in the 12 Midwest states were invited to complete an online anonymous questionnaire. The questionnaire incorporated information of the participants' demographics and educational/clinical backgrounds and MIH-focused questions.

Specific aim 2 & 3 (Chapter 3 and 4): The aims of these studies were to determine the prevalence, severity, and associated sociodemographic determinants of Molar Incisor Hypomineralization (MIH) and Hypomineralized Second Primary Molar (HSPM). A calibrated examiner screened eligible Indiana schoolchildren. Ghanim et al index was used for examining the First Permanent Molars (FPMs), Permanent Incisors (PIs), and Second Primary Molars (SPMs). All teeth present were also examined for dental caries using the ICDAS index. Demographic data were obtained from parents' consents. Zip code area public water supply system was used to obtain data on fluoride level in the subjects' water system from the Center for Disease Control and Prevention database.

CHAPTER 2: U.S. PEDIATRIC DENTISTS' PERCEPTION OF MOLAR INCISOR HYPOMINERALIZATION

2.1. Introduction

Molar incisor hypomineralization (MIH) is defined as a spectrum of developmental qualitative hypomineralization enamel defects affecting the first permanent molars (FPMs) with or without involvement of the permanent incisors. These defects are distributed in an asymmetrical fashion and have discernible variations in severity ranging from demarcated white, yellow, or brown opacities to severe defects with post-eruption disintegration of enamel (Weerheijm, Jalevik et al. 2001, Weerheijm 2003). The condition was formally designated as "Molar Incisor Hypomineralization (MIH)" in 2001 and has attained growing attention in the scientific community ever since. A recent comprehensive analysis of 70 prevalence studies of MIH showed that the problem is very common with a global estimate of around 14% and highest prevalence from South America and Spain (Zhao, Dong et al. 2018). MIH poses extensive oral health challenges (Weerheijm, Jalevik et al. 2001, Weerheijm 2003), and substantial psychological and economical dental treatment burdens (Leppaniemi, Lukinmaa et al. 2001, Jalevik and Klingberg 2002, Ghanim, Manton et al. 2012) for affected children and their families. MIH affected teeth also pose substantial challenges for dental practitioners (Weerheijm and Mejare 2003, Crombie, Manton et al. 2008, Ghanim, Morgan et al. 2011, Kopperud, Pedersen et al. 2016, Silva, Alhowaish et al. 2016). Although one of the pioneer reviews on the management and diagnosis of MIH was published by US scholars (Mathu-Muju and Wright 2006), most of the continuing worldwide growing attention of MIH has been from outside the US. The available US published reports on prevalence of

enamel opacities have been limited to data of the primary dentition (Slayton, Warren et al. 2001). However, scholarly enquiries into the existence of MIH among child populations in the US and whether US dentists are cognizant of the problem are few or nonexistent (Weerheijm 2008). To date and to the best of my knowledge, no attempt had been made to answer these questions in the United States. Thus, this study aims at investigating the level of perception of the MIH problem among pediatric dentists practicing in the US Midwest, determining their insight of the problem's frequency in their practice, and their diagnostic, clinical challenges and management strategies of MIH.

2.2. Methods

2.2.1. Institutional Review Board Approval

Ethical approval was obtained from the Indiana University Institutional Review Board (study IRB number 1610874604).

2.2.2. Target Population and participant recruitment

The Midwest region of the United States –one of the four geographic regions defined by the United States Census Bureau- consists of twelve states occupying the north central United States [Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin] (United States Census Bureau 2015, February 9). After attaining appropriate AAPD authorization, my target population, determined from the AAPD's 2016-2017 Membership Directory, comprised all pediatric dental practitioners who were listed as active or life AAPD members in any of the 12 states of the Midwestern region. To maintain uniformity and limit responses to clinicians who have practiced or are actively practicing pediatric

dentistry in the US, affiliate, associate, international and students' AAPD membership categories were excluded from my sample. As per AAPD definition, affiliate and associate members of the AAPD constitute a wide range of dental professionals including but not limited to foreign-qualified pediatric dentists who may or may not be practicing pediatric dentistry. Email invitations to take part in the study enclosed a link to read the study information sheet and if interested to fill in the questionnaire. The survey was completely anonymous; the investigator obtained a complete list of potential participants' full names, their emails, and state of practice as they appear on the AAPD directory. However, no individual identification appeared on the completed questionnaires and it was not possible to identify participants or link individual answers to a specific participant. Two consequent reminders were sent four and eight weeks after the initial emailing. The online version of the questionnaire was accessible through a secure web application (REDCap™). REDCap™ is an electronic data capture tool for building and managing online surveys and databases hosted at the Indiana Clinical and Translational Sciences Institute (Indiana CTSI).

To ensure concealment, all data were unidentified, and all information collected from the questionnaire was kept confidential and stored in a password protected electronic format.

2.2.3. Data Collection Instrument

The data collection instrument was adopted from the questionnaire utilized in previous studies, with minor modifications. Based on these previous studies (Weerheijm and Mejare 2003, Crombie, Manton et al. 2008, Ghanim, Morgan et al. 2011), the questionnaire was comprised of two main sections: the first section covered demographics, education background, and clinical practice characteristics. The second

section included questions over knowledge and perceptions of MIH's estimated prevalence, incidence, diagnosis, etiology, clinical challenges and restorative options in MIH management. In the demographics section, information about the participant's gender, age, and dental education background were gathered. Questions over the characteristics of the participant's practice as a pediatric dentist - such as the location of the primary practice, involvement in academia, and the average number of pediatric patients examined per workday- were also included in this section. The introductory question about MIH knowledge included a typical clinical picture of a first permanent molar and a permanent incisor with MIH (Figure 1.1.)

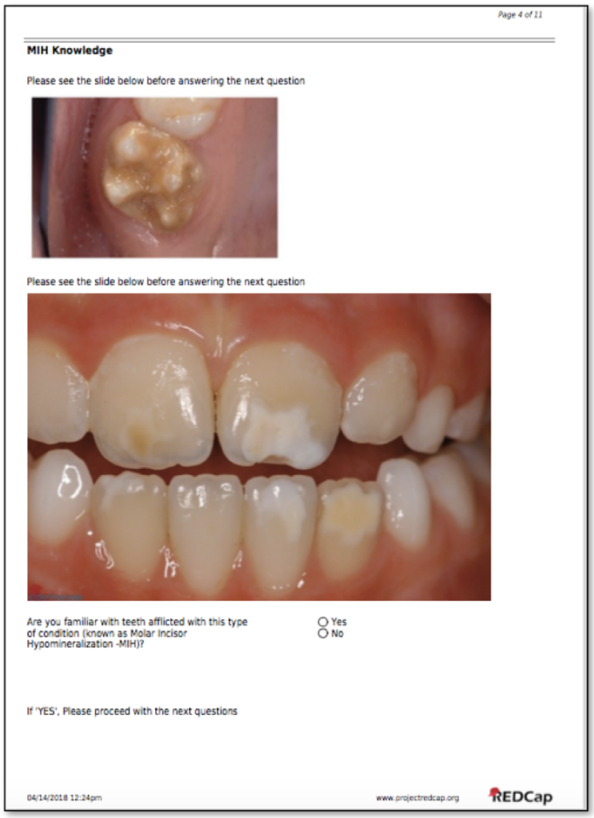
The second section of the questionnaire was further divided into four subcategories. The first category included the participant's views on diagnosis of MIH, confidence in diagnosing MIH, and conditions that they may consider challenging to differentiate from MIH. The second subcategory involved questions over the participant's personal estimation of prevalence and severity of hypomineralized first permanent molar and second primary molars in their clinical practice. A third subcategory incorporated questions about the participant perception over possible etiological factors implicated in MIH. The list of putative factors included "genetics, chronic medical condition/s of the pregnant mother, antibiotics taken by the pregnant mother, antibiotics/medications taken by child, acute medical condition/s that affect the pregnant mother, acute medical condition/s that affect the child, preterm birth/birth and delivery complications, environmental contaminants, high fluoride, and others". For each etiological factor, the participant selected one of the following answers: "yes", "no", "maybe" or "I don't know".

The MIH- related risk factors question was designed as “check all that apply” question. The fourth subcategory explored the most common clinical challenges that face participants in management of MIH and their decisions on restorative material when treating MIH affected molars.

2.2.4. Data analysis

Data were entered into an Excel spreadsheet and analyzed using SAS version 9.4 (SAS Institute Inc., Cary, NC). Descriptive statistics were used to describe the characteristics of the study participants. Associations between survey items were analyzed using chi-square tests. Results at an alpha level<0.05 were considered statistically significant.

Figure 1.1. MIH clinical pictures impeded within the MIH knowledge domain of the Redcap questionnaire



2.3. Results

2.3.1. General descriptive characteristics of study respondents

A total of 975 active and life members of the American Academy of Pediatric Dentistry (AAPD) distributed across the 12 Midwest states were sent email invitations to take part in the study. Seven questionnaires failed to deliver to the recipient, and a total of 251 surveys were returned, yielding an overall response rate of 26%.

Table 2.1. illustrates the study participants' descriptive characteristics. More than 40% of the survey participants completed their pre-doctoral training/qualification (DDS) and their postgraduate (PG) pediatric dentistry training/qualifications between 1990 and 2009 (n=123, 46% and n=117, 43% respectively). A negligible number of participants did not receive formal postgraduate training in pediatric dentistry (n=5, 2%). The majority of participants were board certified in pediatric dentistry (73%), practiced pediatric dentistry in a suburban location (70%), had more than 20 pediatric patients (< 18 years old) in a typical workday (71%), and were not involved in academic/teaching posts (75%).

2.3.2. Knowledge, perceived prevalence and incidence of MIH

Nearly all participants were familiar with teeth afflicted with "Molar Incisor Hypomineralization-MIH". More than half and more than one-third of the participants recalled first learning about MIH during postgraduate residency or pre-doctoral DDS trainings, (58% and 34% respectively). The bulk of survey respondents (n=165, 62%) indicated that they observe MIH in less than 10% of their patients but more than one-third (n=94, 35%) perceived the prevalence of MIH to be around10-25% in their clinical practice. The vast majority (90%) were not aware of MIH published prevalence data for the USA, yet 85% believed that MIH is a significant clinical problem that requires

investigation. More than 40% were uncertain if the incidence of MIH has increased over the last 10 years or in the period of their practice. When comparing perceived prevalence of MIH, responses differed significantly per age group, gender, and year of completion of PG pediatric dental residency. For example, the estimated MIH prevalence in the practice decreased with age (i.e. the older age group of respondents was more likely to estimate the MIH prevalence to be less than 10% in their practice) (Table 2.2.).

Perception of MIH incidence over the period of the participants' practice also differed significantly per age group, gender, year of completion of PG pediatric dental residency, and board certification (data not shown). More than three-fourths of participants (n=203, 76%) agreed that comparable defects of the second primary molars were observed less frequently than those of the first permanent molars. Participants who were in the age group 35-55 years and those who were board certified in pediatric dentistry were significantly more likely to report less frequency of defects in the second primary molars (data not shown).

2.3.3. Diagnosis and differentials of MIH

Most respondents affirmed that they were very confident (n=174, 65%) or confident (n=91, 34%) in diagnosing teeth with MIH, although, more than one third (n=100, 37%) had none of the differential diagnoses checked. Participants who checked differential diagnoses found the most difficulty differentiating between MIH and chronological hypoplasia and fluorosis (36% and 34%, respectively) but the least difficulty differentiating between MIH and tetracycline staining (n=2, 1%), Dentinogenesis Imperfecta (n=12, 4%) followed by Amelogenesis Imperfecta (n=41,

15%). Male respondents and those in the older age group (>55 years) were significantly more likely to feel confident when diagnosing MIH teeth (data not shown).

2.3.4. MIH clinical challenges and management

In the respondents' view of the clinical challenges in the management of MIH teeth, the vast majority agreed that the most common clinical challenge was "long-term success of restorations" (n= 201, 79%), followed by "providing adequate restoration" (n=175, 69%), "determining the extent (or margins) of the affected tooth" (n=172, 67%) and "achieving adequate local anesthesia" (n=170, 67%). The least commonly reported clinical management challenges was "diagnosis" (n=27, 11%). Clinical challenges encountered when managing teeth with MIH differed significantly per age group, gender, year of completion of PG pediatric dentistry training, and board certification. For example, factors associated with being more likely to report achieving adequate local anesthesia of MIH teeth as a clinical challenge were age<= 35, female gender, board certification, and residency completion after 1990 (Table 2.3.).

For the restorative management options, stainless steel crowns (SSCs) and composite resins came as the first and second chosen "most used" (n=81, 32% and n=73, 29% respectively) and "sometimes used" (n=154, 61% and n=114, 45% respectively) dental material options by respondents. Resin-modified glass ionomer (RMGI) emerged as the third chosen restorative option followed by Glass Ionomer (GI) in both categories (RMGI: n=54, 22% and n=104, 42%, GI: n= 42,17% and n=102, 41%). Amalgam was the least used restorative option (n=59, 24%), still more than one fifth of the respondents (n= 52, 21%) cited amalgam as a "sometimes used" restorative option for molars with MIH. Cast restoration was largely the most unused restorative option (n=231, 92%),

followed by compomer and amalgam (n=181, 72% and n=136, 52% respectively). Year of residency completion appears to have an effect across multiple restoration types. Residency completion after 1990 was associated with not using cast restorations and compomer, and completion after 2009 was associated with increased use of RMGI. Amalgam or composite resin use did not significantly differ per age group, gender, year of completion of PG pediatric dental residency or board certification status (Table 2.4.).

2.3.5. Etiology and time of insult

There was considerable inconsistency in the respondents' understanding of MIH etiology. Overall, more than 40% of the participants had a response other than 'No' marked for all the ten etiology options assuming that MIH has a multifactorial etiology. Individually, the most common checked etiologies were "acute medical condition/s that affect the involved child" (n=160, 63%) followed by "chronic medical condition/s that affect the mother during pregnancy" (n=124, 49%). Respondents were mostly uncertain about environmental contaminants as a putative aspect involved in MIH etiology (n=113, 45%), followed by "acute maternal illnesses during pregnancy" and "child intake of antibiotics" (n=102, 41% and n=97%, 39% respectively). Exposure to high Fluoride remained the least proposed MIH etiology (n=108, 44%). The first year of life was the most selected timing of insult (n=90, 35%), Followed by "pregnancy to 1st year of life" (n=75, 29%) and "pregnancy to 3rd year of life" (n=47, 18%).

Table 2.1. General descriptive characteristics of the study respondents

Variable	N (%)
Demographics	
Age in years (Mean, SD)	47.5 (13.1)
Participants' gender	Male 143 (53%), Female 126 (47%)
Dental education background	
Year of completion of postgraduate pediatric dentistry training/qualification	Before 1990's: 71 (26%), Between 1990's &2009: 117(43%), After 2009: 81(30%)
Has postgraduate pediatric dental qualification	264 (98%)
Has board certification in pediatric dentistry	197 (73%)
Dental practice characteristics	
Currently practicing pediatric dentistry	260 (97%)
Primary area of practice	Rural 22 (9%), Suburban 179 (70%), Urban 53 (21%)
Average pediatric patients (<18 years) per day	Less than 10 patients: 10 (4%), 10-20 patients: 65 (25%), More than 20 patients:187 (71%)
Involvement in academic post/teaching	66 (25%)

Table 2.2. Comparison between the participants' demographic and dental education characteristics and their perceived prevalence of MIH and HSPM

Demographic characteristics		**Age**				**Gender**		
		<=35	**36-55**	**>55**	**p-value**	**Male**	**Female**	**p-value**
In approximately what percentage of your patients do you observe MIH?	Less than 10%	21 (37%)	76 (60%)	68 (83%)	**0.000***	102 (72%)	63 (50%)	**0.000***
	~ 10% - 25%	33 (58%)	47 (37%)	14 (17%)		38 (27%)	56 (45%)	
	>25%	3 (5%)	4 (3%)	0 (0%)		1 (1%)	6 (5%)	
How frequently do you notice this defect in the second primary molar tooth in comparison to the first permanent molar tooth?	More frequently	1 (2%)	10 (8%)	7 (9%)	**0.042***	12 (9%)	6 (5%)	0.259
	Less frequently	47 (82%)	100 (79%)	56 (68%)		107 (76%)	96 (77%)	
	Uncertain	7 (12%)	6 (5%)	12 (15%)		13 (9%)	12 (10%)	
	The same as for the first permanent molar	1 (2%)	4 (3%)	6 (7%)		7 (5%)	4 (3%)	
Dental education characteristics		**Board certification**				**Year of completion of Pediatric Dental Residency**		
		No	**Yes**	**P-value**		**Before 1990**	**1990-2009**	**After 2009**	**p-value**
In approximately what percentage of your patients do you observe MIH?	Less than 10%	49 (69%)	116 (59%)	0.336		62 (89%)	69 (59%)	34 (43%)	**0.000***
	~ 10% - 25%	20 (28%)	74 (38%)			8 (11%)	43 (37%)	43 (54%)	
	>25%	2 (3%)	5 (3%)			0 (0%)	4 (3%)	3 (4%)	
How frequently do you notice this defect in the second primary molar tooth in comparison to the first permanent molar tooth?	More frequently	6 (8%)	12 (6%)	**0.028***		5 (7%)	12 (10%)	1 (1%)	0.090
	Less frequently	46 (65%)	157 (81%)			49 (70%)	89 (77%)	65 (81%)	
	Uncertain	13 (18%)	12 (6%)			10 (14%)	5 (4%)	10 (13%)	
	The same as for the first permanent molar	4 (6%)	7 (4%)			4 (6%)	5 (4%)	2 (3%)	

* Statistically significant differences (P<.05) using chi-square test.

Table 2.3. Comparison between the participants' demographic and dental education characteristics and the clinical challenges in the management of MIH

Demographic characteristics		**Age**				**Gender**		
		<=35	**36-55**	**>55**	**p-value**	**Male**	**Female**	**p-value**
Diagnosis	Yes	10 (19%)	10 (8%)	7 (9%)	0.108	13 (10%)	14 (12%)	0.596
Achieving adequate local anesthesia	Yes	50 (93%)	92 (75%)	28 (36%)	**0.000***	74 (55%)	96 (80%)	**0.000***
Determining the extent (or margins) of the affected tooth	Yes	39 (72%)	84 (69%)	49 (62%)	0.421	82 (61%)	90 (75%)	**0.015***
Providing adequate restoration	Yes	46 (85%)	83 (68%)	46 (58%)	**0.004***	81 (60%)	94 (78%)	**0.002***
Long-term success of restoration	Yes	51 (96%)	96 (79%)	54	**0.001***	98 (73%)	103 (87%)	**0.006***
Achieving patient comfort	Yes	46 (85%)	87 (72%)	28 (36%)	**0.000***	71 (53%)	90 (75%)	**0.000***
Dental education characteristics		**Board certification**				**Year of completion of Pediatric Dental Residency**		
		No	**Yes**	**p-value**		**Before 1990**	**1990-2009**	**After 2009**	**p-value**
Diagnosis	Yes	7 (10%)	20 (11%)	0.830		4 (6%)	11 (10%)	12 (16%)	0.163
Achieving adequate local anesthesia	Yes	35 (50%)	135 (73%)	**0.000***		22 (33%)	79 (71%)	69 (90%)	**0.000***
Determining the extent (or margins) of the affected tooth	Yes	44 (63%)	128 (69%)	0.336		37 (55%)	84 (76%)	51 (66%)	**0.018***
Providing adequate restoration	Yes	46 (66%)	129 (70%)	0.537		33 (49%)	82 (74%)	60 (78%)	**0.000***
Long-term success of restoration	Yes	51 (73%)	150 (82%)	0.129		41 (61%)	92 (83%)	68 (89%)	**0.000***
Achieving patient comfort	Yes	35 (50%)	126 (69%)	**0.005***		23 (35%)	77 (70%)	61 (79%)	**0.000***

* Statistically significant differences (P<.05) using chi-square test.

Table 2.4. Comparison between the participants' demographic and dental education characteristics and restorative management options of MIH

Demographic characteristics		**Age**				**Gender**		
		<=35	**36-55**	**>55**	**p-value**	**Male**	**Female**	**p-value**
Stainless steel crowns	0 (Not at all used)	3 (6%)	3 (2%)	1 (1%)	0.239	2 (1%)	5 (4%)	**0.029***
	1 (Least used)	1 (2%)	6 (5%)	5 (6%)		8 (6%)	4 (3%)	
	2 (Sometimes used)	39 (72%)	70 (57%)	45 (58%)		72 (54%)	82 (68%)	
	3 (Most used)	11 (20%)	43 (35%)	27 (35%)		52 (39%)	29 (24%)	
Cast restoration	0 (Not at all used)	53 (98%)	116 (97%)	62 (81%)	**0.000***	118 (90%)	113 (94%)	0.194
	1 (Least used)	1 (2%)	2 (2%)	10 (13%)		7 (5%)	6 (5%)	
	2 (Sometimes used)	0 (0%)	2 (2%)	5 (6%)		6 (5%)	1 (1%)	
Resin modified glass ionomer	0 (Not at all used)	5 (9%)	31 (26%)	24 (31%)	**0.012***	31 (24%)	29 (24%)	0.413
	1 (Least used)	3 (6%)	20 (17%)	9 (12%)		17 (13%)	15 (13%)	
	2 (Sometimes used)	32 (59%)	44 (37%)	28 (36%)		59 (45%)	45 (38%)	
	3 (Most used)	14 (26%)	24 (20%)	16 (21%)		23 (18%)	31 (26%)	
Dental education characteristics		**Board certification**				**Year of completion of Pediatric Dental Residency**			
		No	**Yes**	**p-value**		**Before 1990**	**1990-2009**	**After 2009**	**p-value**
Glass ionomer	0 (Not at all used)	28 (42%)	43 (23%)	**0.026***		23 (35%)	24 (22%)	24 (31%)	0.206
	1 (Least used)	6 (9%)	30 (16%)			6 (9%)	20 (18%)	10 (13%)	
	2 (Sometimes used)	25 (37%)	77 (42%)			29 (45%)	42 (39%)	31 (40%)	
	3 (Most used)	8 (12%)	34 (18%)			7 (11%)	23 (21%)	12 (16%)	
Cast restoration	0 (Not at all used)	59 (88%)	172 (93%)	0.261		52 (80%)	105 (96%)	74 (96%)	**0.001***
	1 (Least used)	6 (9%)	7 (4%)			9 (14%)	2 (2%)	2 (3%)	
	2 (Sometimes used)	2 (3%)	5 (3%)			4 (6%)	2 (2%)	1 (1%)	
Resin modified glass ionomer	0 (Not at all used)	23 (35%)	37 (20%)	**0.046***		22 (34%)	30 (28%)	8 (10%)	**0.001***
	1 (Least used)	6 (9%)	26 (14%)			6 (9%)	21 (19%)	5 (6%)	
	2 (Sometimes used)	28 (42%)	76 (41%)			24 (37%)	36 (33%)	44 (57%)	

* Statistically significant differences (P<.05) using chi-square test.

2.4. Discussion

This study is the first to report on U.S. dentists' perception of Molar Incisor Hypomineralization. Recognition of MIH as a clinical problem from the perspective of pediatric dental practitioners is considered a concrete step to explore this problem, especially in regions where the actual population-based estimates of the problem are scarce or non-existing. The study population comprised of pediatric dentists in the Midwestern U.S. region, who were currently practicing pediatric dentistry (active members) or have maintained their AAPD active status for extended time (life members) at the time of administration of the survey. Although there is discernable disparity in pediatric dentists'-to-child ratio across the different U.S. states (Nainar and Feigal 2004), most Midwestern U.S. states share relatively comparable proportions of pediatric dental practitioners to child population with a range of 2.43 (Michigan) to 4.38 (Indiana) and a total average of 3.33 per 100,000 children (Nainar 2007).

The major limitations of my study are the low response rate and potential self-selection bias, which may compromise the ability to generalize my findings. However, compared to the general population, low response is an identified impediment in health professionals' surveys (Flanigan, McFarlane et al. 2008). As recommended, cover letters, multiple follow up reminders, and a personalized second reminders were all used as response enhancement strategies (McColl, Jacoby et al. 2001). The authors believe that issues such as the anonymous nature of the survey precluding the use of a multi-mode administration (phone calling and postal surveys) and possible administrative staff/ "gatekeepers" scrutiny where participants enlisted their business or group practice email

address may have further augmented the non-response rate (Flanigan, McFarlane et al. 2008).

Mirroring the findings from previous studies (Weerheijm and Mejare 2003, Crombie, Manton et al. 2008) conducted exclusively or partially among pediatric dentists, almost all my survey participants acknowledged acquaintance with MIH, and the vast majority agreed on the clinical significance of exploring MIH. However, very mixed responses were uncovered on questions of perceived prevalence, restorative management options, and etiological factors of the MIH defects.

There were remarkable discrepancies among respondents regarding perceived prevalence of MIH in their clinical practice. While the majority of respondents conformed with the fact of unavailability of published prevalence estimates of MIH from the USA, the bulk inclined towards an estimate of less than 10 percent, which is smaller than the up to date MIH average global prevalence (14%) (Zhao, Dong et al. 2018). While this may reflect regional variations of MIH prevalence consistent with the findings from the European survey (Weerheijm and Mejare 2003), further analysis revealed that respondent pediatric dentists who were younger or those who have completed the residency training recently (after 2009) were more likely to report higher estimated prevalence of MIH (between 10%-25%). In agreement with previous reports (Crombie, Manton et al. 2008, Ghanim, Morgan et al. 2011), these findings could further imply that the issue of hypomineralized FPM is actually increasingly emerging; yet a considerable proportion of respondents were uncertain about the subject of escalating incidence of MIH. The findings likewise could reveal alleged existence of MIH providing partial answers to questions whether MIH exists among U.S. child population. In fact, a recent

analysis has projected the MIH prevalent cases to be the highest in high income countries like the USA (Schwendicke, Elhennawy et al. 2018). Nevertheless, the conclusions of my study reflect only the clinician's personal perceptions of the MIH problem and don't supersede the imperative demand for parallel population-based epidemiological surveys of MIH from the USA.

Gender differences in caries diagnostic and management approaches among clinicians may help explain the same gender differences observed in this study (Riley, Gordan et al. 2011). However, the pronounced influence of participants' other demographics (i.e. age) and their educational background characteristics on their perceived diagnostic and management abilities for teeth with MIH might hint at the wide variations in education and teaching practices in pediatric dentistry residency programs (Casamassimo, Berlocher et al. 2009), the inherent variations of defects' frequency in the participants own practice, and the length of their clinical experiences.

As opposed to diagnosing teeth with MIH, U.S. clinicians reported they were more challenged by providing adequate and long-term restorations of teeth affected by MIH, consistent with their European, Australian/ New Zealander, South American, and Middle Eastern counterpart clinicians (Weerheijm and Mejare 2003, Crombie, Manton et al. 2008, Ghanim, Morgan et al. 2011, Gambetta-Tessini, Marino et al. 2016, Kopperud, Pedersen et al. 2016). Rigorous restorative guidelines of MIH-affected teeth based on substantial and long-term clinical evidence are greatly deficient (Lygidakis 2010), which might have contributed to the noticeable inconsistency observed among respondents in choosing restorative material for FPM with MIH. A recent review has proposed that the estimated mean annual failure rates of restorative materials used in management of teeth

with MIH were highest for glass ionomer and amalgam restorations and lowest for indirect restorations, SSCs, and composite restorations (Elhennawy and Schwendicke 2016). Likewise, and consistent with findings from another survey (Crombie, Manton et al. 2008), there was marked agreement among my survey respondents on preferring SSCs and composite resins. Despite their high failure rate, GI and RMGI were frequently adopted as interim restorations before definitive restorations or extraction of MIH teeth (Fayle 2003, William, Messer et al. 2006, Lygidakis 2010) and their use in MIH affected teeth was mostly a preference of general practitioners and dental auxiliaries rather than pediatric dentists in other similar studies (Crombie, Manton et al. 2008, Hussein, Ghanim et al. 2014, Gambetta-Tessini, Marino et al. 2016). Because the defect severity and eruption status of MIH affected teeth were not sought when constructing the question of the restorative material option of molar teeth with MIH, the considerable fraction of pediatric dentist respondents identifying RMGIs and GI after composite resin and SSC might very likely reflect their adoption of interim restoration strategy before definite treatment. The relatively recent introduction and improved characteristics of RMGI (Croll and Nicholson 2002) might also explain their remarkably magnified use among those who have recently completed their residency training (after 2009). Nevertheless, the unexpected percentage (more than one-fifth) who have cited amalgam as an occasional restorative option of MIH molars remain unwarranted and contradictory to findings from other countries (Crombie, Manton et al. 2008, Hussein, Ghanim et al. 2014, Gambetta-Tessini, Marino et al. 2016, Silva, Alhowaish et al. 2016), but coincide with the overall current U.S. clinicians' inclination towards the continued use of amalgam restorations (Bakhurji, Scott et al. 2017).

The plausibility of MIH being a multifactorial condition, with systemic, environmental and genetic components, is now generally accepted (Crombie, Manton et al. 2009, Silva, Scurrah et al. 2016, Teixeira, Andrade et al. 2018). A recent systematic review (Silva, Scurrah et al. 2016) has highlighted the ample implication of early childhood illnesses in the etiology of MIH as opposed to the weaker evidence of prenatal and perinatal factors. Remarkably, fewer respondents indicated that maternal prenatal and perinatal disturbances are potential MIH risk factors in comparison to postnatal (childhood illnesses), coinciding with the aforementioned suggestions and with findings from earlier surveys (Crombie, Manton et al. 2008, Ghanim, Morgan et al. 2011). The perplexity encountered by participants further on the involvement of genetics and environmental contaminants as alleged MIH risk factors agrees with the conflicting and preliminary findings in the literature (Kuscu, Caglar et al. 2009, Jedeon, De la Dure-Molla et al. 2013, Jeremias, Pierri et al. 2016). Unexpectedly, most respondents drifted towards underrating the timing of insult occurrence to around pregnancy and first year of life, negating the longer enamel mineralization of FPMs period that could extend to an average of three years after birth (Logan and Kronfeld 1933).

CHAPTER 3: PREVALENCE OF MOLAR INCISOR HYPOMINERALIZATION AND OTHER ENAMEL DEFECTS AND ASSOCIATED SOCIODEMOGRAPHIC DETERMINANTS IN INDIANA, USA

3.1. Introduction

Molar incisor hypomineralization (MIH) is a spectrum of developmental qualitative hypomineralization enamel defects affecting the first permanent molars and permanent incisors (Weerheijm, Jalevik et al. 2001, Weerheijm, Duggal et al. 2003). These defects are increasingly becoming an oral health problem around the globe. The initial studies on MIH were predominantly conducted in European countries (Koch, Hallonsten et al. 1987, Alaluusua, Lukinmaa et al. 1996, Weerheijm, Jalevik et al. 2001). South American countries have an extensive, but relatively recent history, of published MIH prevalence estimates with an estimated pooled prevalence of around 18%, placing South America as the continent with the highest MIH prevalence estimate globally (Zhao, Dong et al. 2018). Similar epidemiological data from the North American region was unattainable because corresponding reports have been sparse with only two published prevalence estimates of MIH from Mexico City (Gurrusquieta, Nunez et al. 2017) and Milwaukee, Wisconsin (Davenport, Welles et al. 2019).

Despite the lack of published data exploring the prevalence estimates of hypomineralized first permanent molars in the United States, regional and super-regional projection burden analysis of MIH cases has indicated that high income populations, such as in North America, had the highest prevalence in 2015/2016 (Schwendicke, Elhennawy et al. 2018). The possibility of disagreement regarding the burden of the MIH problem within the USA was also disclosed among American pediatric dentists practicing in U.S.

Midwest (Tagelsir, Dean et al. 2018) who had completed their residency training after 2009 as opposed to their former peers.

With the acceptability of the multifactorial context of MIH (Teixeira, Andrade et al. 2018), factors such as regional, sociodemographic, and ethnic risk indicators of MIH remain largely undetermined, mostly because of the lack of supporting evidence (Jeremias, de Souza et al. 2013, Ghanim, Bagheri et al. 2014, Tourino, Correa-Faria et al. 2016), and the scarcity and conflicting race and ethnicity data from multi-ethnic cohorts (Mahoney and Morrison 2009, Ng, Eu et al. 2015). Thus, extrapolation of the MIH problem within the American context is unquestionably crucial and would unravel many queries about the existence and the magnitude of the problem in North America in general, and the United States in particular. The objectives of this analysis were to determine the prevalence, clinical characteristics, and severity of MIH in a cohort of schoolchildren in the state of Indiana, USA. A secondary objective was to identify potential demographic, racial and regional differences associated with MIH.

3.2. Methods

3.2.1. Institutional review board approval

The study was approved by the Indiana University Institutional Review Board (IRB expedited protocol 1703753377R001 and exempt protocol 1907102161).

3.2.2. Subjects consenting process and recruitment methods

The subjects were either recruited under the umbrella of a school-based dental sealant program (exempt protocol 1907102161) or as part of an independent school recruitment (expedited protocol 1703753377R001). According to the recruitment method and following IRB regulations, parents/guardians of the independent school recruitment

method completed the verbal consenting process over the phone. Parent/guardians of the school-based dental sealant program completed standard written consent forms. Regardless of the recruitment method, all parents/guardians were asked to sign and return a copy of the consent form before the day of clinical examination via the school nurse or class teacher. Consenting and recruitment processes were conducted both in English and Spanish and parents/guardians were given the option to indicate English or Spanish as their preferred language of communication. For completion of the phone consenting procedures, native speaking consenters were appointed for this purpose.

The study subjects were recruited and examined between August 2017 and May 2019. Children were included in the study if they met all of the study eligibility criteria: were a resident of the state of Indiana, between the age of 6-15 years, their parents/guardians had completed a consenting procedure, including signed written approval to be part of the study; and had at least two FPMs fully erupted at the time of examination. Children who had fixed orthodontic appliances at the time of examination or those who didn't comply with the examination procedures were excluded from the study. Children with syndrome-related generalized enamel defects and those with Amelogenesis Imperfecta were also excluded from participation.

*3.2.3. Examination procedure*s

A single trained and calibrated examiner (TAA) performed all dental examinations. First Permanent Molar (FPMs) and Permanent Incisors (PIs) were examined for demarcated and diffused opacities and other enamel defects using the modified Developmental Defects of Enamel and EAPD (Ghanim et al. index) criteria suggested by Ghanim et al., 2015 (Ghanim, Elfrink et al. 2015). Additionally, all teeth

present were examined for dental caries using ICDAS. The codes of ICDAS are explained in detail in Table A.4.

All examination procedures were performed at the school premises on portable dental units under standardized conditions. Before examinations, every participating subject was given a toothbrush and instructed to brush their teeth. No toothpaste was used during the pre-examination tooth brushing. Examination was performed using a mouth mirror, blunt explorer, and a source of artificial light (Zeon^{TM} Endeavour portable LED headlight system, light intensity 34-68 lumens, Orascoptic, Wisconsin, USA). Large debris were removed with the help of a cotton roll or gauze if necessary. Teeth were examined wet for dental enamel defects. Data were collected on an assigned and coded paper folder for each subject. The data were then transferred into a secured electronic database.

3.2.4. Diagnostic indices

The recently proposed index that integrates both the elements of the EAPD criteria and the modified index of developmental defects of enamel (mDDE index) has been adopted in this study (Ghanim, Elfrink et al. 2015). The index incorporates scoring of other enamel defects (diffuse opacity and hypoplasia), has a reasonable validity and reliability (Ghanim, Marino et al. 2019), and categorizes the extension of the MIH defect (less than 1/3, at least 1/3 but less than 2/3, or at least 2/3 of the tooth surface involved). Table A.2. explains further on the scores and description of Ghanim et al., index. 2015.

3.2.5. Examiner's calibrations

Calibration exercises for MIH and ICDAS were finalized before the start of the study. The MIH calibration session was conducted by a benchmark examiner and

consisted of reviewing materials and a training manual focusing on MIH and its most recent diagnostic criteria and other developmental defects of enamel (Ghanim, Elfrink et al. 2015, Ghanim, Silva et al. 2017). The 7- point scoring system employed is a modified index adopted from the "Judgement criteria for MIH in epidemiologic studies" reported by Weerheijm et al in 2003(Weerheijm, Duggal et al. 2003). Calibration sessions included training on the MIH clinical scoring and lesion extension criteria. The short data set form was employed where only the first permanent molars (FPM), permanent incisors (PI) and second primary molars (SPMs) were considered for grading.

The same examiner (TAA) performed the examinations for dental caries. Calibration exercise was conducted by an ICDAS –trained benchmark assessor with at least 70 natural teeth on models with a good mix of the full range of ICDAS scores. Scoring was conducted in two consequent sessions with a time interval of at least two days between the readings.

The intra-examiner and inter-examiner agreement Kappa coefficients for MIH clinical status scores, MIH defect extension, and ICDAS scores were 0.89 and 0.83, 0.83 and 0.75, and 0.82 and 0.78 respectively.

3.2.6. Data collection: demographics and other covariates

The demographic information including date of birth, race and ethnicity, subject's city of residence, county, and zip code were available from the subject's informed consent and/or electronic dental records. For water fluoridation information, the specific zip code area of each participating subject was located on an Indiana map from the site https://www.unitedstateszipcodes.org/. Using the data from the survey of public water supply service areas in Indiana (report available from the Indiana State Department of

Health, 1981), each subject's zip code area public water supply system was matched on corresponding maps and retrieved. Subsequently, data on fluoride level in the subjects' water system were retrieved from the Center for Disease Control and Prevention My Water's Fluoride database available at https://nccd.cdc.gov/DOH_MWF/Default/Default.aspx. If more than one water supply system was available for the specific zip code, then the averages of water fluoridation were calculated.

3.2.7. Statistical methods

Data were analyzed using SAS version 9.4 (SAS Institute Inc., Cary, NC). Descriptive statistics and exact 95% confidence intervals were calculated for the MIH prevalence estimates. Chi-square tests were used to evaluate associations of subject characteristics with the prevalence of MIH and other enamel defects. A 5% significance level was used for all tests. The following analyses were limited to surfaces with MIH defects. Wilcoxon Rank Sum tests were used to compare tooth surfaces and tooth types for differences in MIH severity scores. The associations of age, number of MIH-affected surfaces, and number of MIH-affected teeth with MIH defect severity and extension were evaluated using generalized linear mixed models for ordinal outcomes. Post-study power calculations, assuming a 5% significance level, showed that the study had 80% power to detect a 24% difference in MIH between White and Black participants and a 16% difference between Whites and Hispanics.

3.3. Results

3.3.1. Response rate

Recruitment within the dental sealant outreach program included a total of 305 children out of 536 schoolchildren seen within the predetermined study period and meeting all eligibility criteria, generating a response rate of 56.9%. For the independent school recruitment, 168 out of 990 (16.9%) schoolchildren returned the consents, 45 of the 168 (26.8%) parents/guardians completed the phone consenting process and returned consents to the schools, and 32 subjects (19.0%) were available on the days of the examination.

3.3.2. Description of the sample

The total sample comprised three hundred thirty-seven subjects with a mean age of 9.1(+/-1.7) years. More than half of the subjects (n=177, 52.5%) were between 6-8 years old and more than two-thirds self-identified as non-Hispanics White (n=223, 66.2%). One third of the population included in the study (n=103, 30.6%) had no medical insurance and more than half of the study subjects (n=198, 58.8%) had Medicaid.
The sample included schoolchildren from thirteen different counties of Indiana, six in Southern Indiana, five in central Indiana, and two in northern Indiana. The majority of the subjects lived in rural/mixed Indiana counties (n=235, 69.7%), where the population is between 40,000 to 100,000 and the population density is 100-200 people per sq./mile. Most of the subjects resided in areas with optimal water fluoridation (0.7 ppm fluoride). Table 3.1. and Table 3.2. illustrate the descriptive characteristics of the study sample and the distribution of enamel defects

3.3.3 Prevalence of MIH and other enamel defects (AED): overall and per demographics, geographic location, water fluoridation and caries status

The Molar Incisor Hypomineralization- ALL group (MIH-ALL) was defined as subjects with at least one FPM or at least one FPM and one PI with demarcated opacity or its clinical consequences (clinical status criteria score 2 -6 of the Ghanim et al., index). Of the entire sample, 43 subjects had MIH-with either at least one FPM only or one FPM and one PI affected (MH-ALL 13%, 95% CI 9%-17%). Of those affected, 19 subjects (MIH-FPM+PI 6%, 95% CI 3%-9%) had at least one FPM and at least one PI with demarcated opacity or its clinical consequences (clinical status criteria score 2 -6 of Ghanim et al., index).

Age, race/ethnicity, and gender were not significantly associated with prevalence of any of the MIH groups (MIH-ALL, MIH-FPM only, or MIH-FPM+PI). Prevalence estimates were also not significantly associated with the type of insurance, region within Indiana, living in urban or rural Indiana, or living in an area with optimal water fluoridation (Chi-square test, $p>0.05$).

The Any Enamel Defect (AED) group included any subject with at least one FPM/PI with demarcated opacity or its clinical consequences (FPM/PI with clinical status criteria score 2 -6 of Ghanim et al., index), and/or Diffuse Opacity/Fluorosis (FPM with clinical status criteria score 11 of Ghanim et al., index), and/or any other enamel defect of the FPMs, PIs, or SPMs. Within the whole study population, more than half of the subjects had at least one index tooth with AED (n=176, 52%, 95% CI 47%-58%). Subjects living in an area with water fluoridation more than 0.7 ppm were more likely to have AED than subjects who lived in areas with optimal or suboptimal water fluoridation

(P<0.05) [OR: 2.7 (1.0-7.0) >0.7 ppm vs 0.7ppm, 9.9 (1.6-61.6) >0.7ppm vs <0.7 ppm, and 3.7 (0.8-18.2) 0.7ppm vs <0.7ppm].

Race/ethnicity (non-Hispanic Black) was also significantly associated with higher prevalence estimates of AED (p<0.01), [ORs: 3.1 (1.3-7.6) Black vs White, 2.4 (0.9-6.4) Black vs Hispanic, 1.1 (0.3-4.8) Black vs Others, 1.3 (0.8-2.2) Hispanic vs White, 2.1 (0.6-7.4) Others vs Hispanic, 2.7 (0.8-8.9) Others vs white]. Table 3.3. gives an overview of the association of MIH alone and collectively (AED) with the possible sociodemographic confounders). Caries status was not different in subjects with AED and those without AED. No significant differences in caries status were identified between subjects with MIH-ALL, MIH-FPM only, or MIH-FPM+PI and those without MIH (Chi-square tests, p>0.05). The mean dmfs+DMFS total caries of the study population was not different between those with MIH and those without MIH (8.40±SD 8.48, and 7.19±SD 7.15, p=0.48 respectively) (Table 3.4.).

3.3.4 Defect severity distribution and extension of the MIH

Teeth and surfaces: Overall, molars were more affected than incisors (n=187/226, 82.7%). Mandibular molars (n=101/187, 54.0%) were more affected than maxillary molars, but maxillary incisors (n=26/39 affected incisors, 66.7%) were more affected than mandibular incisors. The most affected molar surfaces were the occlusal surfaces (n=90/ 187) followed by the buccal surfaces (62/187). Buccal surfaces were the most affected in incisors (n=36/39, 92%).

Severity distribution per tooth type: MIH Score 2-demarcated opacity was the most prevalent defect severity in both affected FPMs (n=80/187 teeth, 42.8%) and affected PIs (n=31/39 teeth, 79.4%). The second most common defect severity in affected

FPMs was MIH score 4-atypical restoration (n=64/187, 32.2%). Maxillary FPMs had higher frequency of score 2-demarcated opacity (40/86, 47%) and higher frequency of score 4- atypical restorations (34/86, 40%) than mandibular FPMs (score 2: 40/101, 40%, score 4: 30/101, 30%), however, maxillary and mandibular molars did not have significantly different severity scores (p=0.729). Figure 3.1. illustrates subjects with different defect MIH severity scores of the PIs and FPMs.

Maxillary incisors had higher percentage of score 2 (24/26, 92%) as opposed to mandibular incisors (8/13, 62%). Maxillary incisors had significantly lower severity scores than mandibular incisors (p=0.027).

Severity distribution per tooth surface: Of all the affected surfaces, MIH Score 2-demarcated opacity on the buccal surfaces of FPMs (n=35/62 surfaces, 56%), and the buccal surfaces of PIs (32/36 surfaces, 89%) were the most prevalent surface defect severity.

Buccal surfaces of FPMs had higher frequency of score 2-demarcated opacity (35/62, 56%) but lower frequency of score 4-atypiacl restorations (10/62, 26%) than the occlusal surfaces of FPMs (score 2: 31/90, 35%, and score 4: 33/90, 37% respectively). Buccal surfaces of FPMs had significantly lower severity scores than occlusal surfaces of FPMs (p=0.008). Palatal surfaces of FPMs were not different from buccal (p=0.059) or occlusal (p=0.949) surfaces of FPMs.

Facial surfaces of PIs had higher frequency of score 2-demarcated opacity than the lingual surfaces of PIs (32/36, 92% and 0/3, 0% respectively). However, facial surfaces of incisors had significantly lower severity scores than lingual surfaces of

incisors ($p<0.001$). Further explanation of MIH severity and extent distribution in molars and incisors are shown in figure 3.2. and figure 3.3.

3.3.5 Association of MIH defect severity and extension with age/age group, number of MIH-affected surfaces, and number of MIH-affected teeth

Age was not associated with MIH defect severity. A higher number of MIH-affected surfaces (but not teeth) was associated with having atypical restoration / missing due to MIH. Higher age and a higher number of MIH-affected surfaces were associated with worse MIH extent (Table 3.5.).

Figure 3.1. Study subjects presenting with (A) demarcated opacities of PIs (FPMs of the same subject had similar presentation) (B) post-eruptive enamel breakdown of a mandibular FPM, and (C) Atypical restoration of a mandibular and maxillary FPMs

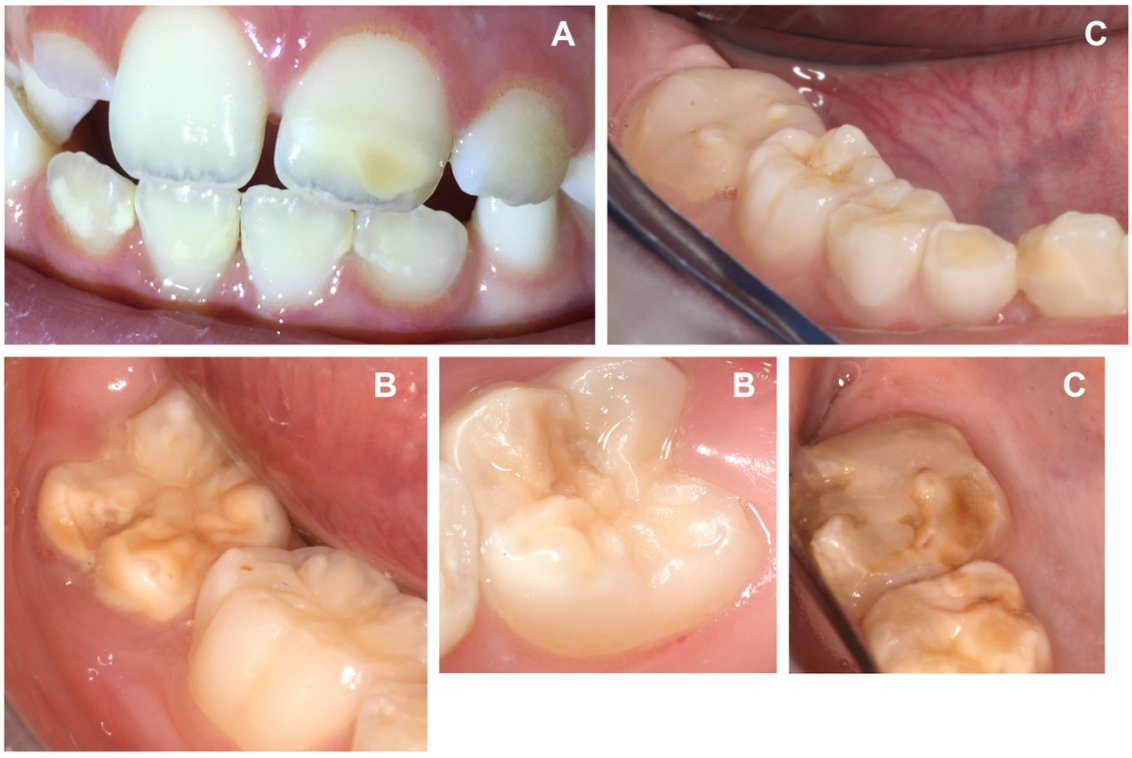


Figure 3.2. Percentage distribution of MIH defect severity scores in FPMs and PIs. Maxillary and mandibular molars did not have significantly different severity scores (p=0.729), while maxillary incisors had significantly lower severity scores than mandibular incisors (p=0.027)

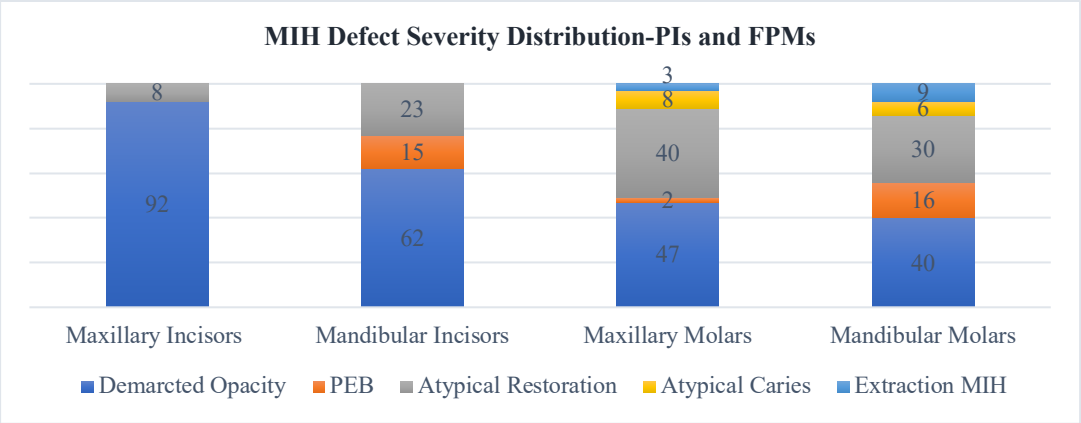


Figure 3.3. Percentage distribution of MIH defect extension in relation to defect severity in FPMs

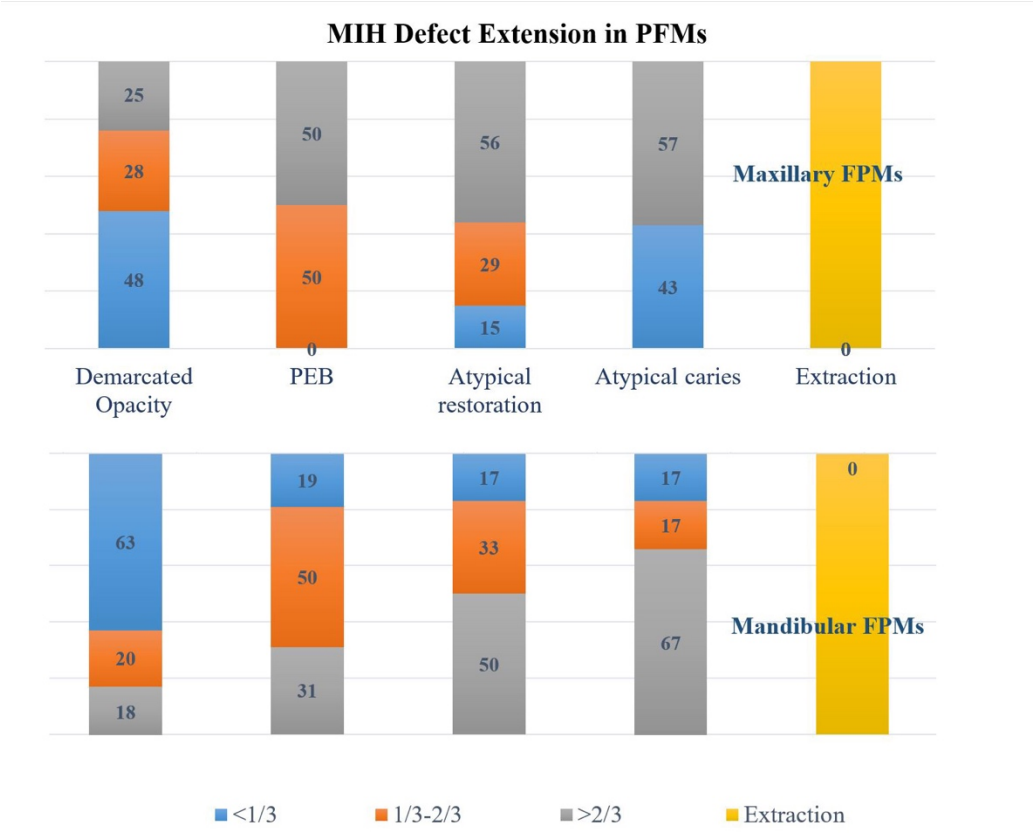


Table 3.1. Descriptive results of the study population

Variable	N (%)
Demographics	
Age (years)	Mean (SD) 9.1 (1.7), Range 6.2-14.9 6-8 177 (52.5%) 9-11 142 (42.1%) 12-15 18 (5.3%)
Participants' gender	Female 169 (50.1%) Male 168 (49.9%)
Race/ethnicity	White 223 (66.2%) Hispanic/Latino 72 (21.4%) Black 27 (8.0%) Other (including biracial/multiracial) 15 (4.5%)
Insurance status	
Medicaid No Medicaid Private insurance Insurance data not available	198 (58.8%) 103 (30.6%) 12 (3.6%) 24 (7.1%)
Geographical distribution	
Indiana county	Bartholomew 61 (18.1%), Boone 42 (12.5%), Brown 18 (5.3%), Crawford 22 (6.5%), De Kalb 8 (2.4%), Greene 12 (3.6%), Gibson 10 (3.0%), Johnson 25 (7.4%), Marion 31 (9.2%), Marshall 66 (19.6%), Monroe 6 (1.8%), Montgomery 16 (4.7%), Shelby 20 (5.9%)
Urbanization	Rural mixed 235 (69.7%) Urban 62 (18.4%) Rural 40 (11.9%)
*Water fluoridation**	
0.7 ppm More than 0.7 ppm Less than 0.7ppm	297 (90.3%) 23 (7.0%) 9 (2.7%)
Caries status	
Any caries	288 (85.5%)
Enamel caries (ICDAS II code 2-3)	223 (66.2%)
Dentine caries (ICDAS II code 4-6)	156 (46.3%)
DMFS+dmfs equal or > than 1	288 (85.5%)

Table 3.2. Frequency distribution (n, %) of enamel defects within the study population

Enamel defects (EDs)	**N (%)**
No EDs	133(40%)
No EDs (SPMs not evaluable)	29(9%)
Diffuse Opacity/Fluorosis and combinations	
DO/Fluorosis only	100(30%)
DO/Fluorosis (SPMs not evaluable)	18(5%)
DO/Fluorosis+HSPM	6(2%)
Molar Incisors Hypomineralization and combinations	
MIH only	22(7%)
MIH (SPMs not evaluable)	4(1%)
MIH+DO	13(4%)
MIH+DO (SPMs not evaluable)	4(1%)
MIH+HSPM	1(0.3%)
MIH+DO+HSPM	1(0.3%)
Hypomineralized Second Primary Molar	
HSPM only	6(2%)

Table 3.3. Prevalence of MIH alone and collectively with other enamel defects (AED) as percentage distribution and 95% Confidence Interval (CI): overall and per demographics, geographical region, water fluoridation, and dental caries

		N (%, 95% Confidence Interval for %)			
		MIH-All	**P Value**	**AED***	**P value**
Overall		43 (13%, 9%-17%)	-	176 (52%, 47%-58%)	-
Demographics					
Age group	6-8	25 (14%, 9%-20%)	0.24	91 (51%, 44%-59%)	0.70
	9-11	14 (10%, 5%-16%)		77 (54%, 46%-63%)	
	12-15	4 (22%, 6%-48%)		8 (44%, 22%-69%)	
Gender	F	24 (14%, 9%-20%)	0.43	94 (56%, 48%-63%)	0.21
	M	19 (11%, 7%-17%)		82 (49%, 41%-57%)	
Race/Ethnicity	White	34 (15%, 11%-21%)	0.23	107 (48%, 41%-55%)	**0.04**
	Hispanic/Latino	8 (11%, 5%-21%)		39 (54%, 42%-66%)	
	Black	1 (4%, 0%-19%)		20 (74%, 54%-89%)	
	Others including multiracial	0 (0%, 0%-23%)		10 (71%, 42%-92%)	
Insurance status					
	Medicaid	22 (11%, 7%-16%)	0.71	101 (51%, 44%-58%)	0.93
	No Medicaid	17 (17%, 10%-25%)		56 (54%, 44%-64%)	
	Private	3 (25%, 5%-57%)		7 (58%, 28%-85%)	
Geographical distribution					
Urbanization	Rural mixed	30 (13%, 9%-18%)	1.00	119 (51%, 44%-57%)	0.06
	Urban	8 (13%, 6%-24%)		40 (65%, 51%-76%)	
	Rural	5 (13%, 4%-27%)		17 (43%, 27%-59%)	
Region	North	11 (15%, 8%-25%)	0.78	32 (43%, 32%-55%)	0.09
	Central	17 (13%, 8%-20%)		79 (59%, 50%-67%)	
	South	14 (11%, 6%-18%)		63 (51%, 42%-60%)	
Water fluoridation					
	0.7 ppm	37(12%, 9%-17%)	0.22	153 (52%, 46%-57%)	**0.02**
	More than 0.7 ppm	5 (22%, 7%-44%)		17 (74%, 52%-90%)	
	Less than 0.7ppm	0 (0%, 0%-34%)		2 (22%, 3%-60%)	
Caries status					
	Any caries	39 (14%, 10%-18%)	0.30	151 (52%, 46%-58%)	0.86
	Enamel caries (ICDAS II code 2-3)	25 (11%, 7%-16%)	0.23	111 (50%, 43%-57%)	0.21
	Dentine caries (ICDAS II code 4-6)	23 (15%, 10%-21%)	0.31	80 (51%, 43%-59%)	0.75
	DMFS+dmfs equal or > than 1	39 (14%, 10%-18%)	0.30	151 (52%, 46%-58%)	0.86

Table 3.4. Caries experience (Mean +/- SD) of MIH affected molars and non-affected molars

	MIH (More than one FPM affected) N=37	MIH (One FPM affected) N=6	No MIH N=294	p-value
dmfs+DMFS Total Caries	9.05 (8.79)	4.33 (4.89)	7.19 (7.15)	0.32
dmfs+DMFS Enamel Caries	1.92 (4.21)	1.00 (1.10)	1.63 (2.83)	0.26
dmfs+DMFS Dentine Caries	1.63 (2.83)	1.67 (2.25)	1.92 (4.21)	0.74
Sealants	0.70 (1.65)	1.00 (1.55)	1.14 (1.71)	0.16

Table 3.5. Median (25th Percentile – 75th Percentile) of age, number of MIH-affected surfaces, and number of MIH-affected teeth by MIH severity and extent

	MIH Severity					
	Score 2-Demarcated Opacities	Score 3-Post-Eruptive Breakdown	Score 4-Atypical Restoration	Score 5-Atypical Caries	Missing due to MIH	p-value
Age	7.9 (7.2-9.2)	8 (7-9.1)	9.1 (9-10.1)	9.6 (7.7-9.8)	11.6 (11.6-12.3)	0.15
# MIH-affected Surfaces	5.5 (3-9)	5 (4-9)	10 (8-12)	4 (2-5)	12 (11-12)	**0.033**
# MIH-affected Teeth	4 (2-5)	3.5 (3-5.5)	5 (4-6)	2 (2-4)	4 (4-4.5)	0.26
	MIH Extent					
	Less than 1/3 affected	At least 1/3 but less than 2/3	At least 2/3 affected	Missing due to MIH	p-value	
Age	8.5 (7.3-9.8)	8.1 (7.1-9.1)	9 (7.9-9.9)	11.6 (11.6-12.3)	**<.001**	
# MIH-affected Surfaces	6 (4-9)	7 (4-10)	8 (4-10)	12 (11-12)	**0.003**	
# MIH-affected Teeth	4 (3-5)	5 (3-5)	4 (3-5)	4 (4-4.5)	0.08	

3.4. Discussion

The study reports on the prevalence of Molar Incisor Hypomineralization and other enamel defects including diffuse opacities/fluorosis among a cohort of USA schoolchildren. This study, which included children from thirteen different counties across the state of Indiana, together with a recent report from Wisconsin (Davenport, Welles et al. 2019), represent the initial reports on MIH among US schoolchildren. The overall prevalence of MIH in my sample (13%) was slightly higher than the one reported from Wisconsin (10%). However, my findings are still marginally lower than the global prevalence estimates of MIH (14%) (Schwendicke, Elhennawy et al. 2018, Zhao, Dong et al. 2018). The same holds true when comparing my study finding with the MIH prevalence estimates from the southern parts of the Americas (Mexico:15.8% and Brazil:19.9%) (Gurrusquieta, Nunez et al. 2017, Zhao, Dong et al. 2018). My data on MIH prevalence were also consistent with the estimates of younger pediatric dentists practicing in the U.S. Midwest who participated in another study conducted by my group. Results of that study showed that more recent graduates or younger pediatric dentists perceived that MIH was present in more than 10% of the patients in their clinical practice (Tagelsir, Dean et al. 2018). With the availability of both Indiana and Wisconsin MIH prevalence estimates, but bearing in mind that at least three prevalence figures are required (Zhao, Dong et al. 2018) to have pooled MIH estimate of the problem for a specific region, it might be premature to conclude that the U.S. Midwest region has an MIH estimated prevalence on the lower end of the spectrum.

My study has some limitations that need to be acknowledged. The power of my study only allowed us to estimate modest differences. The age range is large which might

have skewed the MIH defect severity of the study population. Moreover, Indiana children in the upper socioeconomic status were underrepresented in the study population, mainly due to the fact that schools with an overriding concentration of children with higher socioeconomic status were only recruited through the independent school recruitment, which yielded a very low response rate. This might have underestimated the overall prevalence of MIH of the study sample as higher odds of having MIH have been reported in children of families with higher annual income in Brazil (Biondi, Cortese et al. 2011, Teixeira, Andrade et al. 2018). The authors believe, that among other impediments in the recruitment of US schoolchildren in epidemiological studies, having a dental home/private dentist might be a reason for non-participation in school-based dental studies.

Consistent with the conclusions from a comprehensive analysis of seventy MIH studies (Zhao, Dong et al. 2018), gender was not associated with MIH when scrutinized as a risk factor. On the other hand, pronounced geographical differences in MIH prevalence estimates were evident in reports from Bosnia and Herzegovina (Muratbegovic, Zukanovic et al. 2008) and Japan (Saitoh, Nakamura et al. 2018), yet my estimate was not significantly different either on city, county, region, urbanization, or water fluoridation levels.

When examining the prevalence of MIH alone as opposed to the collective prevalence of enamel defects of the FPMs, PIs, and SPMs, being a resident in an area with higher than optimal water fluoride concentration was significantly associated with higher prevalence estimates of these enamel defects. This particular finding in my study might be comprehensible since diffuse opacities/fluorosis were the most prevalent enamel

defect (>30%) within this cohort of children. My data didn't report on fluorosis severity, but the general prevalence of fluorosis in the study cohort is consistent with the 1986-1987 NIDR and the 1999-2004 NHANES US fluorosis data, although not with the more recent 2011- 2012 NHANES fluorosis prevalence data (Neurath, Limeback et al. 2019). The significant association of water fluoridation with the collective prevalence of enamel defects in the study cohort and the lack of such significant relationship between MIH prevalence estimate and water fluoridation not only agree with the established biological mechanism of fluorosis, but also confirms the notion that MIH is not associated with excessive fluoride intake during tooth development, congruent with previous Swedish (Koch 2003) and British (Balmer, Toumba et al. 2012) MIH reports.

Except for the Malay ethnicity as a risk determinant of MIH (Ng, Eu et al. 2015), the literature lacks evidence associating race/ethnicity with MIH. Despite the pronounced health-related disparities of Latinos and African Americans –the two largest ethnic minority groups in the USA- and the established evidence associating early childhood illness with higher odds of MIH (Fatturi, Wambier et al. 2019), my findings were not able to support the presumption that being Latino or Black/African American is a risk determinant of MIH. This coincides with the Wisconsin US MIH data which failed to identify race/ethnicity as a predilection determinant of MIH despite the overrepresentation of Hispanic/Latino in their sample (Davenport, Welles et al. 2019). The lack of race/ethnicity association with MIH reinforces the postulation that MIH is rather a multifactorial condition that involves the interaction of genetic vulnerability with the exposure to systematic and environmental insults (Jeremias, Pierri et al. 2016). Only when analysis was conducted collectively for enamel defects including MIH and diffuse

opacity/fluorosis was race/ethnicity a significant risk determinant of having higher prevalence of enamel defects. Although the issue of race/ethnicity as a risk for fluorosis in U.S. children remains controversial (Arora, Kumar et al. 2018), my findings agree with previous results where Black/African Americans had higher odds of having fluorosis than other races (Butler, Segreto et al. 1985).

Although MIH has been associated with higher prevalence of dental caries (da Costa Silva, Ortega et al. 2017), my data failed to detect differences in enamel and dentine caries between children with MIH and those without MIH. While this finding corresponds with those mainly from low caries risk populations (Dietrich, Sperling et al. 2003, Heitmuller, Thiering et al. 2013), my study population had noticeably high dmfs/DMFS scores, exceeding the national average of U.S. children and adolescents (Slade, Grider et al. 2018). This is an anticipated finding considering the low-income and underserved composition of the study population. Further studies exploring MIH and dental caries among USA children with different sociodemographic makeup might reveal different outcomes.

Age was not associated with MIH defect severity, contrasting other reports (Leppaniemi, Lukinmaa et al. 2001, Kevrekidou, Kosma et al. 2015). However, my study outcomes indicate older age and having an MIH tooth with higher number of affected surfaces were positively associated with the defects extending over larger tooth surface, supporting previous findings (da Costa-Silva CM 2011, Fragelli, Jeremias et al. 2015) where the implementation of early diagnostic and secondary preventative measures are considered fundamental to intervene with the progression of MIH defects.

CHAPTER 4: HYPOMINERALIZED SECOND PRIMARY MOLAR (HSPM) AND OTHER ENAMEL DEFECTS IN INDIANA, USA: PREVALENCE AND ASSOCIATED SOCIODEMOGRAPHIC DETERMINANTS

4.1. Introduction

Demarcated hypomineralization of the second primary molars is a qualitative developmental enamel defect, presumed to be related to post-secretory disturbance of amelogenesis basically around the intrauterine period and up to 12 months postnatally (Suckling 1989). Second primary molars (SPMs) commence enamel mineralization somewhat earlier than first permanent molars (FPMs) and permanent incisors (PIs), but generally at very analogous time frames (Elfrink, Moll et al. 2014).

The exact etiology of HSPM lacks supporting evidence (Silva, Scurrah et al. 2016), but environmental insults rather than genetic influences have been strongly related to the etiology of HSPM (Silva, Kilpatrick et al. 2019).

Demarcated hypomineralization of the second primary molars (HSPM) share numerous connotations with that of hypomineralization of the first permanent molars (MIH) and while the burden of HSPM is less investigated and appears less conspicuous than that of MIH, both defects share comparable variations in prevalence estimates from different parts of the world (HSPM: 5%-20%)(Elfrink, Schuller et al. 2008, Temilola, Folayan et al. 2015, Gambetta-Tessini, Marino et al. 2019, Silva, Kilpatrick et al. 2019). In general, demarcated hypomineralization opacities of the second primary molars are not a recent manifestation, but in the early 2000's the literature described demarcated opacities of molars and their concomitant clinical consequences as post-eruptive breakdown and atypical restorations utilizing the European Academy of Pediatric

Dentistry diagnostic criteria (Weerheijm, Duggal et al. 2003). The earliest studies to explore HSPM prevalence using these diagnostic criteria were from the Netherlands (Elfrink, Schuller et al. 2008, Elfrink, ten Cate et al. 2012). From the Americas, studies exploring the problem were limited to two South American countries, Brazil and Chile (da Silva Figueiredo Se, Ribeiro et al. 2017, Gambetta-Tessini, Marino et al. 2019). Although there are no similar epidemiological studies employing the EAPD diagnostic criteria from any of the North American countries, the early literature from the United States have reported on demarcated opacities of the primary teeth in Iowan (Slayton, Warren et al. 2001) and Californian (Nation, Matsson et al. 1987) children, mostly using the established modified developmental defect of enamel (mDDE) index.

Together with the dearth of prevalence data of HSPM from the USA, and the fact that the earlier reports from the USA (Nation, Matsson et al. 1987, Slayton, Warren et al. 2001) have used indices that disregarded progressive clinical manifestations of the defects, it is imperative to have epidemiological data elaborating on the substantial burden of the defect among U.S. children. Therefore, the aim of this report was to determine the prevalence of HSPM and to describe associated sociodemographic risk determinants in a group of U.S. school- and preschool children in the state of Indiana.

4.2. Methods

4.2.1 Ethical approval

The study received appropriate approval from the Indiana University Institution Review Board (exempt 1907102161 and expedited 1703753377R001 approvals).

4.2.2. Subjects' recruitment

The study was conducted as part of a larger project examining MIH, HSPM, and other enamel defects of schoolchildren in Indiana. Study subjects were preschool children

from Head Start programs and schoolchildren from selected public elementary schools seen as part of a community outreach school program within the state of Indiana. Head Start programs are U.S. federally funded preschool programs that incorporate children under five years mainly from low-income families. The recruitment methodology is explained in detail in Chapter 3. In brief, recruitment was either conducted as part of an outreach dental sealant program or independently. Eligibility for participation included the following: children 3 years and older, residents of the state of Indiana, had at least one SPM for evaluation, and had returned a signed informed consent.

4.2.3. Examiner calibration and diagnostic criteria

Details on the calibration and the Kappa coefficients for the intra-examiner and inter-examiner agreements are detailed in Chapter 3. Demarcated opacities were recorded when a white/creamy or yellow/brown change of enamel translucency was observed. Demarcated opacities with associated disintegration of enamel with demarcated irregular enamel borders were recorded as post-eruptive breakdown. Hypoplasia was differentiated by the presence of pits, grooves, or linear deficiency of enamel thickness, mostly with smooth borders of enamel (Weerheijm 2003). Additionally, Buccal/facial, lingual/palatal, and occlusal surfaces of all teeth present at the time of examination were evaluated for dental caries using the ICDAS criteria.

4.2.4. Examination procedures and data collection

All examinations were conducted during the regular school day within the school premises. Each child was seated on a portable dental chair for examination. Together with the classroom lighting, a portable head light (Zeon^{TM} Endeavour portable LED headlight system, light intensity 34-68 lumens, Orascoptic, Wisconsin, USA) was used.

Examinations were carried out with a regular dental mirror and a blunt dental explorer on wet teeth surfaces. Before the dental exam, each subject was given a toothbrush and instructed to brush their teeth for at least one minute. Large debris that were still retained on the tooth surface were removed with a cotton roll or a gauze.

Sociodemographic data were collected from patients' questionnaire and electronic dental records. Residence and zip code information were allocated from informed consents. Water fluoridation data of each subject were extracted by exploiting the subject's zip code information matched to the data from the survey of public water supply service areas in Indiana (report available from the Indiana State Department of Health, 1981). Then the fluoride level of the specific water supply system was consequently retrieved from the Center for Disease Control and Prevention My Water's Fluoride database available at https://nccd.cdc.gov/DOH_MWF/Default/Default.aspx. Subjects who had arrived within one year in the US were excluded from the water fluoride analysis.

4.2.5. Statistical analysis

Data were analyzed using SAS version 9.4 (SAS Institute Inc., Cary, NC). Descriptive statistics and exact 95% confidence intervals were calculated for the HSPM prevalence estimates. Chi-square tests were used to evaluate associations of subject characteristics with HSPM prevalence. Generalized linear mixed models for ordinal outcomes were used to evaluate the associations of age, number of HSPM-affected surfaces, and number of HSPM-affected teeth with HSPM defect severity and extension; these analyses were limited to surfaces with HSPM defects. Post-study power calculations, assuming a 5% significance level, showed that the study had 80% power to

detect a 16% difference in HSPM between White and Black and a 10% difference between White and Hispanic.

4.3. Results

4.3.1. Response rate

Of the total 654 schoolers and preschoolers seen as part of the community outreach dental program during the school year 2018-2019, 392 subjects (60%) met all inclusion criteria and agreed to be part of the study. For the independent school recruitment, 45 subjects (4.5%) out of the 990 invited subjects completed the consenting process, and 31 subjects (3%) were available for the examination, making a total sample of 423 subjects.

4.3.2. Description of the sample

Four hundred and twenty-three subjects were included in the exams conducted between April 2018 and May 2019 across public elementary schools and preschools as part of an outreach dental school sealant program in the state of Indiana, USA. The mean age was 7.6 (+/-2.2) years and more than half of the study subjects (n=254, 60.0%) were in the age group 6-9 years old. The study had almost equal percentages of male and female participants (females n=208, 49.2%) and the majority self-identified as non-Hispanics whites (n=286, 67.6%). All the schools involved in the study were identified as Title I schools where there is a hefty concentration of low-income students. One third of the sample (n=129, 30.5%) had no medical/dental coverage, and more than sixty percent were covered by Medicaid (n=257, 60.8%).

The study population was recruited from equal numbers of counties in Central Indiana (six counties, n=187, 44.2%) and Southern Indiana (six counties, n=147, 34.7%) with a smaller portion from Northern Indiana (two counties, n=89, 21%). Most of the subjects lived in rural/mixed Indiana (n=235, 69.7%), had optimal water fluoridation (n=368, 86.9%), and had enamel or dentine caries (n= 338, 80%). Table 4.1. and Table 4.2. illustrate the descriptive characteristics and enamel defect distribution of the study population.

4.3.3. Prevalence of HSPM and other enamel defects of the SPMs: overall and per demographics, geographic location, water fluoridation and caries status

The Hypomineralized Second Primary Molar (HSPM) group- included any subject with at least one Second Primary Molar (SPM) with demarcated opacity or its clinical consequences (clinical status criteria score 2 -6). Of the whole study population, 25 subjects had at least one SPM affected (HSPM 6%,95% CI 4%-9%). Any Enamel Defect of the Second Primary Molar (AED of the SPM) group combines subjects with demarcated opacities and/or any other enamel defects (diffuse opacities and hypoplasia) of at least one SPM (clinical status criteria score 1 -6). Additionally, seven subjects had other enamel defect of at least one SPM with an overall prevalence of 8% (95% CI 5%-11%) of the SPMs. Of all the sociodemographic determinants tested, Chi-Square analyses revealed that race/ethnicity (being other than White, Black, or Hispanic/Latino) was significantly associated with higher prevalence estimates of AED of SPMs but not HSPM prevalence estimates ($p<0.05$) [ORs 2.0 (0.5-8.2) Others vs Black, 6.7 (1.7-26.1) Others vs Hispanic, 5.0 (1.8-14.0) Others vs White, 3.3 (0.8-14.3) Black vs Hispanic, 2.5 (0.8-7.9) Black vs White, 1.3 (0.4-4.2) White vs Hispanic]. Table 4.3. illustrates the

prevalence of HSPM and the overall prevalence of enamel defects of the SPMs and their associated sociodemographic determinants.

4.3.4. Prevalence of enamel defects of the index teeth (SPM and FPM/PI): overall and per demographics, geographic location, water fluoridation and caries status.

When examining the combination of demarcated opacity or its clinical consequences of the SPMs and/or the FPMs/PIs (HSPM+/-MIH group) in this study population, 63 subjects (15%, 95% CI12%-19%) had at least one SPM and/or one FPM/PI with score 2-6 of the mDDE-EAPD diagnostic index. The group of Any Enamel Defect (AED) includes any subject with HSPM, MIH, diffuse opacities of SPM, FPM/PI, and/or other enamel defect of the index teeth (SPM, FPM/PI). 169 of the study population (40%, 95% CI 35%-45%) belonged to the AED group. Neither age group, gender, nor race/ethnicity or any geographical or water fluoridation confounders were significantly related to the prevalence estimates of HSPM+/-MIH. However, dentine caries experience (ICDAS score 4-6) was significantly higher in the HSPM+/-MIH group than in the group without HSPM+/-MIH (P=0.02, OR 1.9 (1.2-3.3). Children in the oldest age group (equal or >10 years) were more likely to have higher prevalence estimates of AED of the FPM/PI, and SPM than children in the younger age groups (P<0.01) [ORs 6.6 (3.1-14.0) equal or >10 years vs 3-5 years, 1.1 (0.6-1.9) equal or >10 years vs 6-9 years, and 5.9 (3.2-11.0) 6-9 years vs 3-5 years].

Subjects who were residents of the central Indiana region had significantly higher prevalence of AED of FPMs, PIs, and SPMs than subjects living in other regions of Indiana (p=0.03) [ORs 2.1 (1.2-3.6) Central vs South, 1.3 (0.8-2.2) Central vs North, and 1.5 (0.9-2.7) South vs North]. Living in areas with water fluoridation more than 0.7 ppm

was significantly associated with higher prevalence estimates of AED of index teeth than children living in areas with optimal or suboptimal water fluoridation ($p<0.01$) [ORs 3.8 (1.5-9.3) >0.7 vs 0.7 ppm, 8.9 (1.9-42.0) >0.7ppm vs <0.7, 2.4 (0.6-8.6) 0.7 ppm vs <0.7ppm]. The mean DMFS/dmfs were not different for subjects with HSPM and those without HSPM (6.76±SD 6.04, and 5.99±SD 6.33 respectively). Table 4.4. shows the prevalence estimate of demarcated opacities of the SPMs and/or FPMs (HSPM+/-MIH) and the overall enamel defect prevalence of index teeth and their associated demographics.

4.3.5. Severity, defect distribution, and extension of HSPM

One hundred and six SPMs were identified as HSPM. Maxillary molars (n=55/106, 51.9%) were more affected than mandibular molars (51/106, 48.1%). All in all, demarcated opacities (score 2) were the most prevalent defect severity of the affected SPMs (36/106, 34%), followed by atypical caries (score 5) as the second most common defect severity (n=27/106, 25%). Twenty (19%) and 17 (16%) SPMs had post-eruptive enamel breakdown and atypical restorations respectively. Six mandibular SPMs (6%) were extracted due to the defect. Occlusal surfaces of the SPMs were the most affected surfaces (n=42/106, 37%), followed by the palatal surfaces (33/106, 31%). However, no significant differences were found between defect severity of affected surfaces (p=0.15 Occlusal vs Buccal, p=0.92 Occlusal vs Palatal, p=0.29 Buccal vs Palatal) or maxillary and mandibular SPMs (p=0.91). Figure 4.1. and 4.2. show the distribution of HSPM defect severity per tooth type and tooth surface. Figure 4.3. describes the extension of the HSPM defect per each category of defect severity.

4.3.6 Association of HSPM defect severity and extension with age /age group, number of HSPM-affected surfaces, and number of HSPM-affected teeth

Age was not associated with HSPM defect severity nor with defect extension ($p>0.05$). A higher number of HSPM-affected surfaces or teeth was associated with having HSPM scores above score 2-demarcated opacity ($p<0.05$). A higher number of HSPM-affected surfaces or teeth was associated with having at least 1/3 of the surface affected by HSPM or missing due to HSPM ($p<0.05$) (Table 4.5.).

Figure 4.1. Percentage distribution of HSPM defect severity of SPMs. No difference in severity score distribution between maxillary and mandibular SPMs (P>0.05)

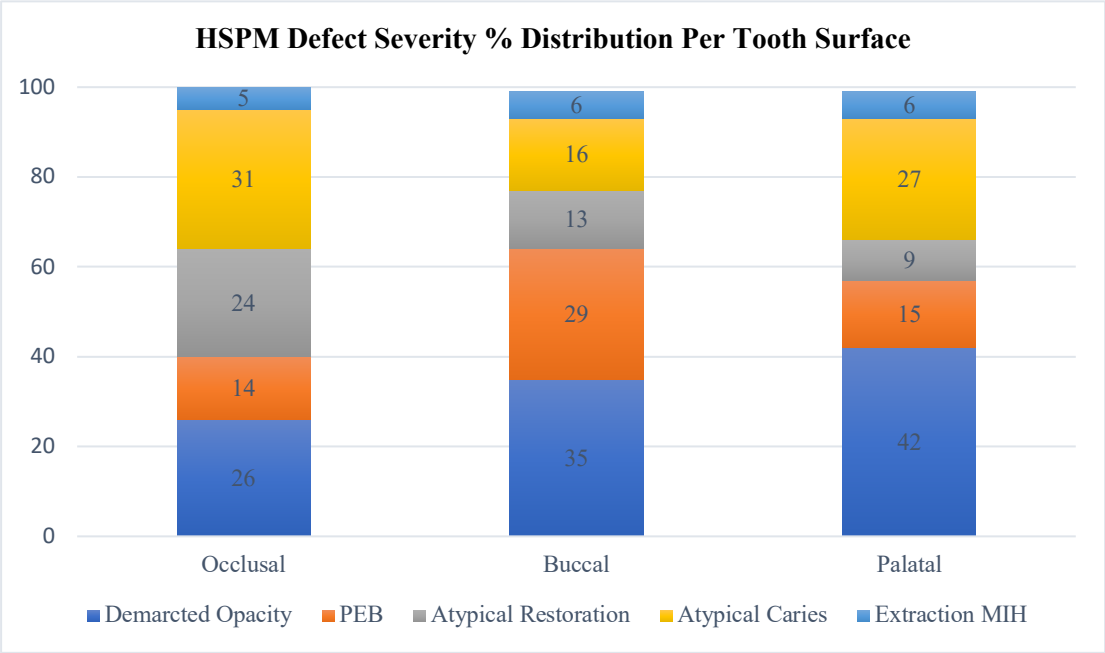


Figure 4.2. Percentage distribution of HSPM defect severity per SPM surface. No difference in defect severity distribution between the different SPM surfaces (P>0.05)

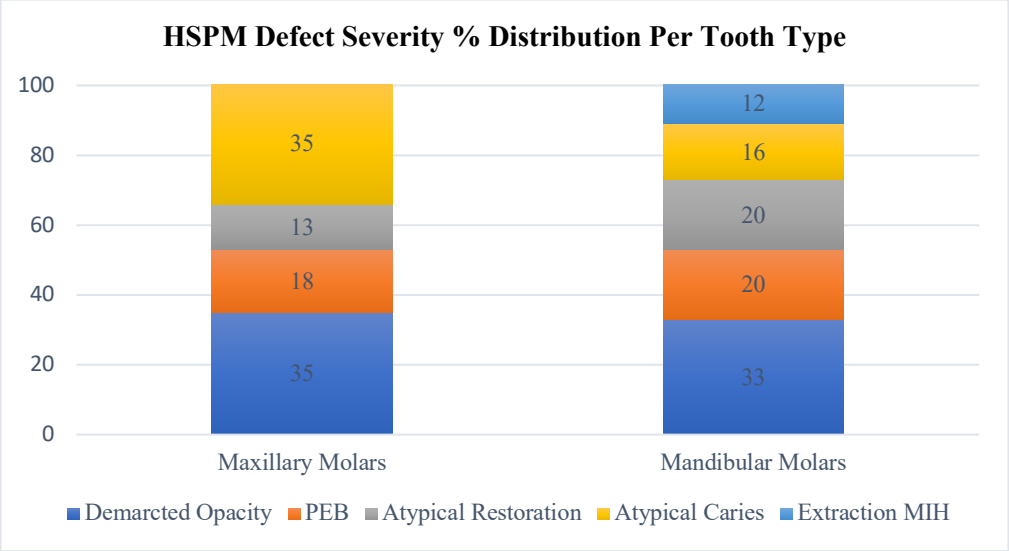


Figure 4.3. Percentage distribution of HSPM defect extension in relation to the defect severity in SPMs.

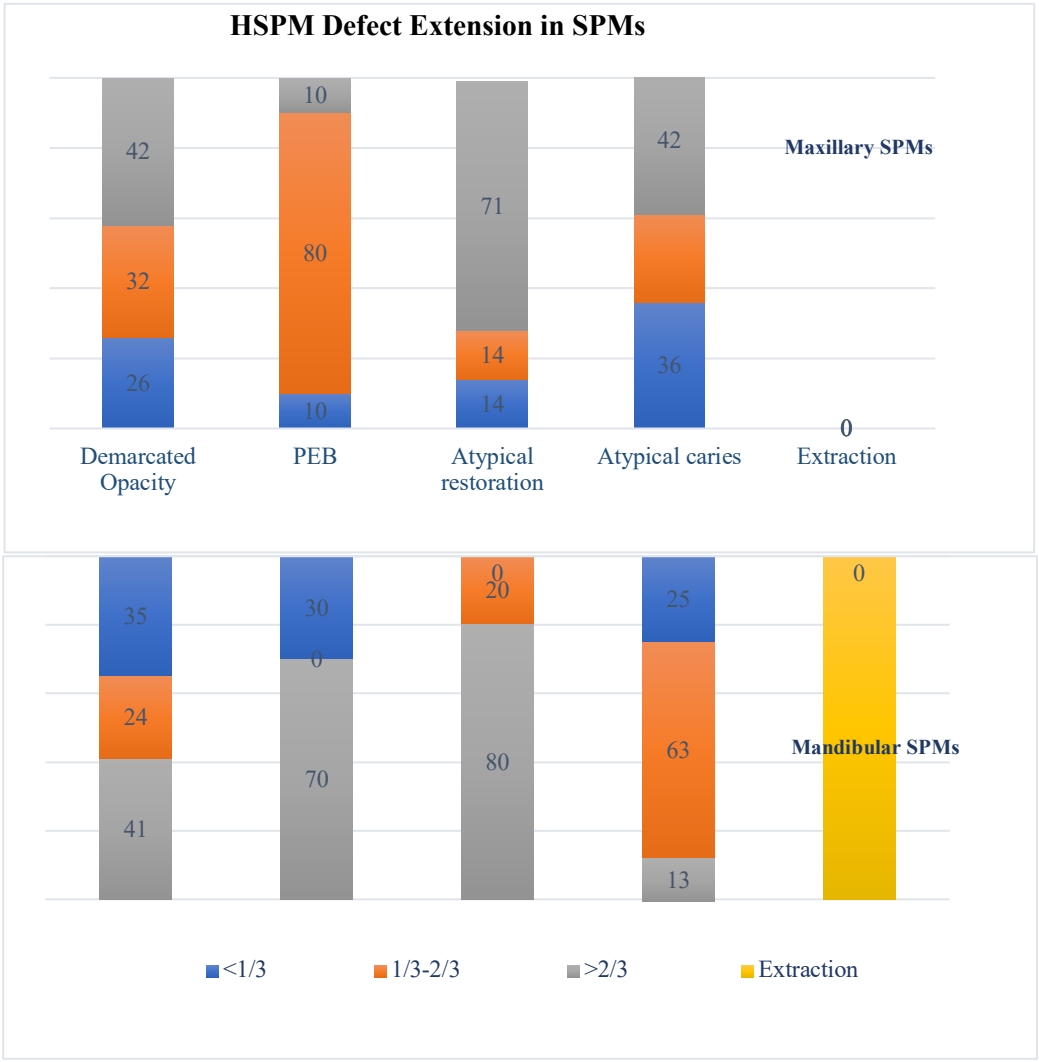


Table 4.1. Descriptive characteristics of the study population

Variable	N (%)
Demographics	
Age (years)*	Mean (SD) 7.6 (2.2), Range 3.0-12.7 3-5 years 104 (24.6%), 6-9 years 254 (60.0%), ≥10 years 63 (14.8%)
Participants' gender	Female 208 (49.2%), Male 215 (50.8%)
Race/ethnicity	White 286 (67.6%), Hispanic/Latino 84 (19.9%), Black 28 (6.6%), Other (including multiracial) 24 (5.7%)
Insurance status	
Medicaid No Medicaid Private insurance Insurance data not available	257 (60.8%) 129 (30.5%) 15 (3.5%) 22 (5.2%)
Geographical distribution	
Indiana county	Bartholomew 56 (13.2%), Boone 42 (9.9%), Brown 23 (5.4%), Crawford 18 (4.3%), De Kalb 16 (3.8%), Greene 18 (4.3%), Gibson 11 (2.6%), Johnson 33 (7.8%), Marion 23 (5.4%), Marshall 73 (17.3%), Monroe 21 (5.0%), Montgomery 25 (5.9%), Shelby 18 (4.3%), Wayne 46 (10.9%)
Urbanization	Rural mixed 294 (69.5%), Urban 77 (18.2%), Rural 52 (12.3%)
Water fluoridation	
0.7 ppm More than 0.7 ppm Less than 0.7ppm	368 (86.9%) 24 (5.7%) 14 (3.3%)
Caries status	
Any caries	338 (79.9%)
Enamel caries (ICDAS II code 2-3)	266 (62.9%)
Dentine caries (ICDAS II code 4-6)	190 (44.9%)
DMFS+dmfs equal or > than 1	338 (79.9%)

Table 4.2. Distribution of Enamel Defects (EDs) of the study participant

Enamel defects (EDs)	**N(%)**
No EDs of SPMs (FPMs/PIs not evaluable)	103(24.3%)
No EDs of SPMs with intact FPMs/PIs	150(35.5%)
No EDs of SPMs with EDs of the FPMs/PIs	138 (32.6%)
EDs of FPMs/PIs	
Diffuse Opacity/Fluorosis (FPMs/PIs)*	122(29%)
Molar Incisors Hypomineralization (FPMs/PIs)*	42(10%)
EDs of the SPMs	
HSPM*	25(6%)
Non-HSPM (other enamel defect of SPMs)*	8(2%)

*with or without combination with other enamel defects

Table 4.3. Prevalence estimates of HSPM and other enamel defects of SPMs as percentage distribution and 95% Confidence Interval (CI): overall and per demographics, insurance status, geographical distribution, water fluoridation, and caries status

		N (%, 95% Confidence Interval for %)			
		HSPM	**P value**	**AEDs of SPMs***	**P value**
Overall		25 (6%, 4%-9%)	-	32 (8%, 5%-11%)	
Demographics					
Age group	3-5	9 (9%, 4%-16%)	0.45	11 (11%, 5%-18%)	0.42
	6-9	13 (5%, 3%-9%)		17 (7%, 4%-10%)	
	≥10	3 (5%, 1%-13%)		4 (6%, 2%-15%)	
Gender	F	15 (7%, 4%-12%)	0.31	17 (8%, 5%-13%)	0.71
	M	10 (5%, 2%-8%)		15 (7%, 4%-11%)	
Race/Ethnicity	White	15 (5%, 3%-9%)	0.11	18 (6%, 4%-10%)	**0.01**
	Hispanic/Latino	3 (4%, 1%-10%)		4 (5%, 1%-12%)	
	Black	3 (11%, 2%-28%)		4 (14%, 4%-33%)	
	Other (including multiracial)	4 (17%, 5%-37%)		6 (25%, 10%-47%)	
Insurance status					
	Medicaid	15 (6%, 3%-9%)	0.14	19 (7%, 5%-11%)	0.22
	No Medicaid	7 (5%, 2%-11%)		10 (8%, 4%-14%)	
	Private	1 (7%, 0%-32%)		1 (7%, 0%-32%)	
Geographical distribution					
Urbanization	Rural mixed	17 (6%, 3%-9%)	0.81	22 (7%, 5%-11%)	1.00
	Urban	4 (5%, 1%-13%)		6 (8%, 3%-16%)	
	Rural	4 (8%, 2%-19%)		4 (8%, 2%-19%)	
Region	North	6 (7%, 3%-14%)	0.60	7 (8%, 3%-16%)	0.27
	Central	9 (6%, 3%-12%)		14 (10%, 6%-16%)	
	South	5 (4%, 1%-9%)		6 (5%, 2%-10%)	
Water fluoridation					
	0.7 ppm	23 (6%, 4%-9%)	1.00	28 (8%, 5%-11%)	0.40
	More than 0.7 ppm	1 (4%, 0%-21%)		3 (13%, 3%-32%)	
	Less than 0.7ppm	0 (0%, 0%-23%)		0 (0%, 0%-23%)	
Caries Status					
	Any caries	22 (7%, 4%-10%)	0.44	27 (8%, 5%-11%)	0.65
	Enamel caries (ICDAS II code 2-3)	17 (6%, 4%-10%)	0.67	21 (8%, 5%-12%)	0.85
	Dentine caries (ICDAS II code 4-6)	16 (8%, 5%-13%)	0.06	18 (9%, 6%-15%)	0.20
	DMFS+dmfs equal or > than 1	22 (7%, 4%-10%)	0.44	27 (8%, 5%-11%)	0.65

Table 4.4. Prevalence estimates of HSPM+/-MIH and other enamel defects of the index teeth (SPMs and FPMs/PIs) as percentage distribution and 95% Confidence Interval (CI): overall and per demographics, insurance status, geographical distribution, water fluoridation, and caries status.

		N (%, 95% Confidence Interval for %)			
		HSPM+/-MIH	**P value**	**AED***	**P value**
Overall		63 (15%, 12%-19%)	-	169 (40%, 35%-45%)	-
Demographics					
Age group	3-5	12 (12%, 6%-19%)	0.15	14 (13%, 8%-22%)	**<.01**
	6-9	45 (18%, 13%-23%)		122 (48%, 42%-54%)	
	≥10	6 (10%, 4%-20%)		32 (51%, 38%-64%)	
Gender	F	38 (18%, 13%-24%)	0.06	91 (44%, 37%-51%)	0.14
	M	25 (12%, 8%-17%)		78 (36%, 30%-43%)	
Race/Ethnicity	White	47 (16%, 12%-21%)	0.64	106 (37%, 31%-43%)	0.08
	Hispanic/Latino	9 (11%, 5%-19%)		34 (40%, 30%-52%)	
	Black	3 (11%, 2%-28%)		17 (61%, 41%-78%)	
	Other (including multiracial)	4 (17%, 5%-37%)		12 (50%, 29%-71%)	
Insurance status					
	Medicaid	33 (13%, 9%-18%)	0.27	93 (36%, 30%-42%)	0.38
	No Medicaid	24 (19%, 12%-26%)		57 (44%, 35%-53%)	
	Private	3 (20%, 4%-48%)		7 (47%, 21%-73%)	
Geographical distribution					
Urbanization	Rural mixed	44 (15%, 11%-20%)	0.76	115 (39%, 34%-45%)	0.52
	Urban	10 (13%, 6%-23%)		35 (45%, 34%-57%)	
	Rural	9 (17%, 8%-30%)		19 (37%, 24%-51%)	
Region	North	15 (17%, 10%-26%)	0.80	31 (35%, 25%-46%)	**0.03**
	Central	24 (17%, 11%-24%)		74 (52%, 44%-61%)	
	South	18 (14%, 9%-22%)		57 (45%, 36%-54%)	
Water fluoridation					
	0.7 ppm	53 (14%, 11%-18%)	0.29	144 (39%, 34%-44%)	**<.01**
	More than 0.7 ppm	6 (25%, 10%-47%)		17 (71%, 49%-87%)	
	Less than 0.7ppm	1 (7%, 0%-34%)		3 (21%, 5%-51%)	
Caries status					
	Any caries	55 (16%, 12%-21%)	0.13	142 (42%, 37%-47%)	0.11
	Enamel caries (ICDAS II code 2-3)	40 (15%, 11%-20%)	1.00	109 (41%, 35%-47%)	0.61
	Dentine caries (ICDAS II code 4-6)	37 (19%, 14%-26%)	**0.02**	80 (42%, 35%-49%)	0.43
	DMFS+dmfs equal or > than 1	55 (16%, 12%-21%)	0.13	142 (42%, 37%-47%)	0.11

Table 4.5. Median (25th Percentile – 75th Percentile) of age, number of HSPM-affected surfaces, and number of HSPM-affected teeth by HSPM severity and HSPM extent

	HSPM Severity					
	Demarcated Opacities	Post-eruptive Breakdown	Atypical restoration	Atypical caries	Missing due to HSPM	p-value
Age	5 (4.6-6.6)	9 (9-9)	8.8 (8.8-8.8)	7.5 (5.6-8.1)	7.5 (7.5-7.5)	0.12
# HSPM-affected Surfaces	4 (2-6)	12 (4-12)	11 (7-11)	6 (3-6)	8 (8-8)	**0.038**
# HSPM-affected Teeth	2 (2-4)	4 (2.5-4)	4 (4-4)	2 (2-4)	4 (4-4)	**0.038**
	HSPM Extent					
	Less than 1/3 affected	At least 1/3 but less than 2/3	At least 2/3 affected	Missing due to HSPM	p-value	
Age	7 (5.5-9.7)	8.1 (4.7-9)	7.2 (4.9-8.8)	7.5 (7.5-7.5)	0.71	
# HSPM-affected Surfaces	2.5 (2-3)	6 (4-7)	6 (6-11)	8 (8-8)	**0.002**	
# HSPM-affected Teeth	2 (2-2)	4 (2-4)	4 (2-4)	4 (4-4)	**0.003**	

4.4. Discussion

In reviewing the literature, this appears to be the first report from the USA on hypomineralized second primary molars (HSPMs) employing the EAPD diagnostic criteria as described by Weerheijm (Weerheijm, Duggal et al. 2003). The diagnostic index used in this study documents not only the prevalence estimates of HSPM but expands to include other enamel defects of the FPMs and PIs when available for evaluation. The study also contributes to my understanding not only of the occurrence but the associated sociodemographic characteristics of HSPM in a group of US preschool (3-5 years) and schoolchildren (6-12 years).

Examining the prevalence estimate of HSPM reported by my study and considering the unavailability of other US reports investigating the prevalence of HSPM strictly following the Weerheijm criteria (Weerheijm, Duggal et al. 2003), makes running any comparison with U.S. studies unfeasible. The earlier U.S. study by Nation et al, 1987 (Nation, Matsson et al. 1987) reported estimates of enamel opacities of 12% in California children attending Loma Linda pediatric dental clinics. Other than the different diagnostic criteria employed in the study, the younger age cohort (3-6 years), the hospital-based recruitment, and inclusion of all primary teeth rather than only the SPMs might have contributed to overemphasizing the prevalence of enamel opacities in that cohort of U.S. children. Slayton and co-investigators (Slayton, Warren et al. 2001) have also reported a high prevalence of isolated enamel opacities of 27% in Iowa. Similar to the deviations of my study from the Californian study (the different diagnostic index and inclusion of all primary teeth), the Iowan study sample incorporated a different socioeconomic

population of relatively high SES, which would warrant careful exploration of their findings to the results of the present study.

Nevertheless, when examining the global estimate of the problem, my HSPM prevalence estimate is analogous to the figures obtained from the only two South American studies (Brazil: 6% (da Silva Figueiredo Se, Ribeiro et al. 2017) and Chile: 5% (Gambetta-Tessini, Marino et al. 2019). My findings also showed comparable HSPM prevalence estimates to those reported among Dutch (Elfrink, Schuller et al. 2008), German (Kuhnisch, Heitmuller et al. 2014), Iraqi (Ghanim, Manton et al. 2013), and Nigerian (Temilola, Folayan et al. 2015) cohorts of children.

Considering the race/ethnicity issue, my study was not able to identify differences between ethnic groups when comparison was limited to the prevalence of HSPM. Out of the negligible number of studies that have explored HSPM ethnic risk factors, a single prospective cohort was able to identify Dutch ethnicity as a risk determinant of HSPM (Elfrink, Moll et al. 2014). It must be emphasized that within the American perspective where people are descendants of diverse multiethnic immigrant groups, we were only able to extract the race/ethnicity variable based on the parents' self-identification rather than based on the country of birth of the mothers as in the Dutch study (Elfrink, Moll et al. 2014). Along the same line of examining the role of race/Ethnicity as a risk determinant, extending the comparison to include all enamel defects of the SPMs, children who were self-identified as other than any of the three ethnic groups (non-Hispanic white, non-Hispanic Black/African American, or Hispanic/Latino) were significantly at risk of having higher enamel defects of the SPMs than those from other ethnic backgrounds. These findings contrast the earlier findings where Black/African

American children were reported to be at higher risk of having enamel defects of primary teeth when compared to Caucasians and Latinos (Nation, Matsson et al. 1987). However, the appraisal of these findings remains hampered by the different goal and methodology of the previous report and entail the imperative need for further inquiry into the role of race/ethnicity as a risk factor in large multi-ethnic cohorts.

Age was not a risk determinant of having HSPMs in my study population, nor was it positively correlated with the severity or the extension of the HSPM defect. This could point out to one of the limitations of the study, where the wide age range may have attenuated the effect of age. Yet, the age effect as a risk determinant was evident when examining the overall prevalence estimates of enamel defects, as the older age groups had significantly higher prevalence of enamel defects (more than 50% in the age group of 10 years or older had enamel defects of at least one index tooth). This is fairly logical as almost one third of the study population had at least one permanent index tooth with fluorosis. These defects would be recorded in the older age groups (six years and older) when permanent index teeth (FPMs/PIs) were present for evaluation.

My study was also incapable of identifying discrepancies in the prevalence estimates of HSPMs based on geographical variables (residence zip code, residence county and region). On the other hand, living in Central Indiana but not in Northern or Southern Indiana was associated with higher odds of having overall prevalence of enamel defects in the study population. The scarcity of U.S. data on enamel defects from Indiana or any of the Midwestern U.S. states creates inadequate room for comparison based on regional variances. However, it has been demonstrated that USA counties have declining fluoridation rates from the most urban to the most rural, implying that children living in

the most urbanized counties have higher access to community water fluoridation than those living in the less urbanized counties (Hendryx, Constance Weiner et al. 2012) and according to the index of Relative Rurality (RR) of the U.S. counties (0: most urban, and 1 most rural)(Waldorf 2007), more number of counties with lower index of Relative Rurality were represented within the Central Indiana region than within Northern or Southern Indiana, which might be a valid explanation why more children in the central region of Indiana had higher overall prevalence of enamel defects. To further augment my previous assumption, children within the study population living in areas with higher than optimal fluoride in drinking water were also notably more likely to have higher overall prevalence of enamel defects than those living in areas with optimal or less than optimal water fluoridation.

The outcome of the present report should be assessed with caution as it might not represent the actual estimation of HSPM, considering the wide age range and the overrepresentation of Indiana schoolchildren with lower socioeconomic background. However, this might also explain the high prevalence of dental caries within the study, where 80% of the children had enamel or dentine caries. When examining the difference in dentine caries experience of children with HSPMs and/or MIH, the conspicuous difference in dental caries was only evident in the group of children with demarcated hypomineralization of molars (FPMs and SPMs) than those without HSPM and/or MIH. This association disappeared when the collective enamel defects (including diffused opacities) were analyzed for the prevalence of caries. The trend of associating demarcated opacities (including MIH and HSPM) and higher prevalence of dental caries have been well documented in permanent teeth (Ellwood and O'Mullane 1994) as well as

in primary teeth (Costa, Silveira et al. 2017). The reverse trend where no correlation was evident between the overall enamel defects and dental caries as apparent in my study might have been enhanced by the high prevalence of diffuse opacities/fluorosis (almost 1/3 of the study population showed signs suggestive of fluorosis in permanent teeth) and the very low prevalence of other non-demarcated opacities and enamel hypoplasia. While these particular findings were in contrast to the outcome of a meta-analysis where all enamel defects, regardless of the type, have been positively associated with dental caries (Vargas-Ferreira, Salas et al. 2015), it is important to acknowledge that more than 85% of this study cohort lived in areas with optimal water fluoridation and most of the diffuse opacities defects observed in the this cohort were within the very mild-mild levels of fluorosis severity, which are recognized to grant resistance to dental caries.

In addition to the high caries experience of my study population, the subjects examined in this study had advanced severity of HSPM, with more than 60% of the affected SPMs showing a severity score higher than demarcated opacity including six extracted mandibular molars due to the hypomineralization defect. While the inclusion of older children in this study might have attenuated the influence of age on the defect severity, this was not the case when defect severity, number of teeth affected, and extension of the defects were scrutinized. My study was able to reveal a positive relationship between the advanced severity with the number of teeth affected as well as with the extension of the defect. This trend of positive association between HSPM defect severity, the number of teeth affected, and the defect size has also been established in younger Melbourne preschool children with an overall low caries experience (Owen, Ghanim et al. 2018).

CHAPTER 5: THE CASE OF INADEQUATE DATA ON MOLAR INCISOR HYPOMINERALIZATION FROM THE USA: ROUTES AND IMPEDIMENTS TO EPIDEMIOLOGICAL SCHOOL-BASED DATA

5.1. Background

The problem of demarcated hypomineralization defects of the first permanent molars (Molar Incisor Hypomineralization-MIH) has been an emerging issue for the oral health professionals around the globe. Almost two decades after the problem of MIH was first described in 2001, the setback of data scarcity on MIH from the USA remains an unsolved challenge. It has been proposed that the lack of effort to obtain data are due to a lack of cognizance of USA dentists and researchers (Weerheijm 2008). And, while there is very little information to refute the assumption that the lack of awareness of U.S. scholars is the cause, this issue has been partially underpowered by the early existence of a comprehensive review from the USA on the diagnosis and management of MIH defects (Mathu-Muju and Wright 2006). Although not generalizable to all US dentists, results from my recent survey (Chapter 2) among a group of pediatric dentists practicing in the Midwest region of the USA (Tagelsir, Dean et al. 2018) revealed that almost all survey participants were aware of this specific enamel developmental defect and that the bulk majority believed that MIH is a significant clinical problem that requires investigation.

5.2. The available data on MIH from the USA

Table 5.1. shows an overview of the MIH data available from the USA, both from school-based surveys and clinical settings. The earliest available set of MIH data (n=340 children) were clinical data collected from four clinic sites affiliated with UT Health at San Antonio in 2014-2015 (Cervantes Mendez MJ, Abudawood S et al. 2015). This was

complemented later to reach a larger sample of 1212 children seen within the same clinical affiliation (Cervantes Mendez MJ, Abudawood S et al. 2016). While these data represent the forerunner estimate of the MIH problem in the USA, recruitment of subjects from a dental- hospital-based population may result in an overestimation of the burden of the problem and is not an accurate reflection of the frequency of the problem within the general population.

5.3. Routes and potential impediments to MIH school-based data collection

5.3.1. Data collected through individual research projects

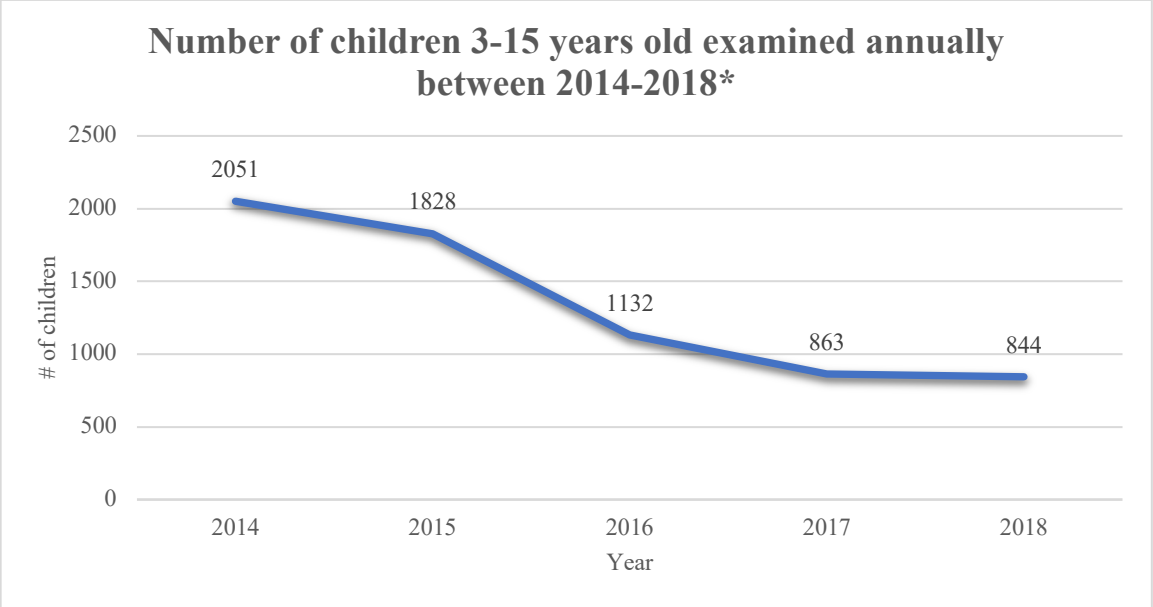
The principal problem in accessing school-based data is the complicated and multi-level recruitment and consenting processes dictated by USA regulations. Large U.S. school districts have research review boards and project proposals would ultimately undergo double reviewing processes both through the affiliated academic institution and the school district review boards. A central challenge to this route of data collection other than the prolonged time required to develop and establish relationships with the targeted schools and to obtain research proposal approvals, is the need for long-term funding and personnel for schools' recruitment, consent delivery and collection, in addition to the onsite projects' assistants and examiners.

5.3.2. Data collected through outreach community-based dental programs and school-based dental sealant programs

Another feasibility issue when collecting MIH prevalence data is driven by the complexity of incorporating calibrated examiners into outreach community dental and/or school-based sealant programs. Many-but not all- of these programs are affiliated with dental schools and collecting such data would offer appropriate teaching opportunities for

the dental students attending these outreach rotations. There are mainly two major setbacks to this route. First, the presence of more than one outreach mobile program within a state/geographic territory, which could prevent the recruitment of all potential subjects. The second major setback of this route is that these mobile outreach programs are designed to serve populations with certain socioeconomic profiles, which would compromise the sample generalizability aspects. Figure 5.1. illustrates the decreasing number of recruited children reflecting a noticeable decline of the consent return rates through a dental school-affiliated mobile outreach program within a 5-year period primarily due to the appearance of other mobile programs operating within the same state.

Figure 5.1. Number of recruited children annually through a dental school-affiliated mobile outreach program within a 5-years period



*total number of recruited children includes annual rechecks

5.3.3. Data of the national oral health surveillances conducted by the state department of health and partner organizations

The Centers for Disease Control and Prevention (CDC) provides funding to several state health departments and national partner organizations to implement activities conducive to improving oral health outcomes; of these, oral health surveillance is a major activity. The National Oral Health Surveillance System (NOHSS), a joint collaboration between CDC's Division of Oral Health and the Association of State and Territorial Dental Directors (ASTDD), includes untreated and treated caries and fissure sealants as the child oral health indicators. Approximately 30 states have updated oral health data surveys conducted in 2010 and later of U.S. third grade schoolchildren. This age group is the recommended age by the European Academy of Pediatric Dentistry for MIH examination for epidemiological purposes. Running MIH dental exams under the umbrella of national oral health surveys has been the case for some of the European MIH data (Dietrich, Sperling et al. 2003, Balmer, Toumba et al. 2012). There are, however, several impediments, if building partnerships and collaborations with the U.S. state oral health departments is sought for the purpose of collecting state/national MIH data. First of all, these surveys collect pre-identified child oral health indicators and lack the workforce capacity and training to conduct any additional dental exams. Other than this fundamental hurdle, the substantial variations between state departments in the availability of funding, the size and the capacity of the examiners team, the standardization of examination protocols, and examiners' calibration represent additional legitimate holdups to this route for MIH data collection.

5.3.4. Data of The National Health and Nutrition Examination Surveys (NHANES)

NHANES is the only national US survey that provides population-representative estimates of fluorosis. This national survey has been scoring fluorosis since 1999 and have demonstrated enormous rise in the prevalence and severity of fluorosis in the 2011-2012 NHANES data. Expanding this survey to include MIH data would entail additional work burden similar to the other suggested routes. However, the assumption that misdiagnosis of MIH as fluorosis was not ruled out as one of the many rationalizations explaining the high rates of fluorosis in the 2011-2012 NHANES data when specific-tooth analysis of the 12-15 years children was carried out (Neurath, Limeback et al. 2019). This would foster the fact that collecting MIH data analogous to fluorosis data through these U.S. national surveys would provide answers at numerous levels.

Table 5.1. School- and clinic-based data on Molar Incisor Hypomineralization from the USA

Author, year	State	Age (years)	Sample (n)	Diagnostic Criteria	Duration of data collection	MIH Prevalence
School-based data						
Davenport M et al., 2019(Davenport, Welles et al. 2019)	Wisconsin	7–12	375	EAPD 2003	December 2014-June 2015	10%
Tagelsir A. et al. 2019(Tagelsir AA, Soto Rajas AE et al. 2019)	Indiana	6-14	266	mDDE-EAPD 2015	April-December 2018	12%
Clinic-based data						
Cervantes Mendez et al., 2015(Cervantes Mendez MJ, Abudawood S et al. 2015)	Texas	6-14	346	EAPD 2003	NA	34%
Cervantes Mendez., 2016(Cervantes Mendez MJ, Abudawood S et al. 2016)	Texas	6-14	1212	EAPD 2003	NA	29%
Mohamed A and Adhia R, 2019(Ahmed Mohamed and Adhia 2019)	New York	7-10	44	EAPD 2003	NA	23%

CHAPTER 6: GENERAL DISCUSSION AND CONCLUSIONS

The problem of Molar Incisor Hypomineralization is a challenging issue at multiple levels; the compromised children oral health, the associated morbidities and the management predicaments faced by the clinicians. The U.S.A remains one of the few countries around the globe with scarce data on MIH. One of my long-term scholarly goals is to positively impact understanding of the MIH problem within the U.S.A perspective, and eventually contribute to unlocking the mystery behind the etiology of the Molar Hypomineralization. This goal would be realizable not only through the data provided in this dissertation, but also through the invaluable experiences gained during the various steps of conceiving and executing these studies.

In Chapter 2, I explored dental professionals' knowledge and perceptions of the MH problem, an area that has never been reported from the US. The survey employed in this study is a simple straightforward tool that has been used widely outside the USA and provides appropriate information about the MH problem from the dental professional's perspectives. Acknowledging the limited ability to generalize the findings of my survey, data from this survey can lay the foundation for a broader survey targeting other categories of oral health professionals and extending it to other geographical regions of the USA. Moreover, the inconsistency encountered in the pediatric dentist's perception of the MH problem might pinpoint the imperative need to explore the teaching practices of developmental enamel defects including MIH across pre-doctoral and postdoctoral USA programs.

In Chapter 3 and Chapter 4, I recruited and screened a cohort of USA school and pre-schoolchildren to investigate the magnitude of the MIH and HSPM. Employing a

single calibrated examiner and adopting the Ghanim et al., index diagnostic index provided the advantages of reporting reliable data and also determining the prevalence of MH in addition to the prevalence of other common enamel defects as fluorosis. The limitations of these studies as the age range selection and the underrepresentation of some racial/ethnic minorities and children with higher socioeconomic profiles could be eliminated in future cohorts to have a broader insight into the MH problem in the USA.

In Chapter 5, The short communication navigates the different routes for collecting MIH school-based data and explains potential impediments to each suggested route, partially based on the experience gained during recruitment of schoolchildren for the epidemiological studies (Chapter 3 and 4).

Based on these studies, we conclude the followings;

Chapter 2: pediatric dentists from the U.S. Midwest who responded to this survey were well aware of the problem of Molar Hypomineralization. However, discrepancies, similar to those reported in previous studies, were pronounced in most aspects of the problem, such as MIH's perceived prevalence, clinical management, restorative material choices, and etiological aspects. Factors such as the participants' demographics and educational characteristics were significantly associated to the different perceptions and approaches reported.

Chapter 3: Nearly one in six children in Indiana has at least one FPM with MIH. None of the demographic determinants were identified as risk factors of MIH. However, children living in areas with water fluoridation > 0.7 ppm or those who were self-identified as non-Hispanic Black had higher odds of having enamel defects in general.

MIH defect extensions were positively associated with age and higher number of affected surfaces.

Chapter 4: In this population of Indiana schoolchildren, HSPM had a prevalence estimate of 6% and an overall enamel defect prevalence of 40% of the examined index teeth (SPMs and/or FPMs/PIs). Caries experience was not different between the group with HSPM and those without HSPM, but dentine caries experience was almost two times higher in the group with demarcated molar hypomineralization (HSPM+/-MIH) than in the group without demarcated molar hypomineralization. Demographic factors as region of residence, water fluoridation, and age group were significantly associated with the overall prevalence of enamel defects of the index teeth, but not with the prevalence estimate of HSPM.

Recommendations for further research

Based on the findings from this dissertation, the following recommendations were developed

- Expanding the scope of research on MIH perception to include USA general practitioners and other specialists in different USA regions.
- The pattern and quality of teaching of DDE enamel in pre- and post- doctoral pediatric dentistry programs in the USA need to be investigated and addressed.
- Expanding the MIH epidemiological surveys to accommodate other USA regional/national data. Within these MIH epidemiological studies, issues like calibration of examiners, cohort selection, and minority representation need to be fully tackled.

APPENDIX

Table A.1. The EAPD judgment criteria for Molar Incisor Hypomineralization

Criteria	Description
Demarcated opacities	A demarcated defect involving an alteration in the translucency of the enamel at occlusal and buccal part of the crown The defects are variable in degree and color: can be white, creamy or yellow to brownish. Defective enamel is of normal thickness with a smooth surface
Enamel disintegration/Post-eruptive enamel breakdown (PEB)	A defect that indicates loss of initially formed surface enamel after tooth eruption. The loss is often associated with a pre-existing demarcated opacity
Atypical restorations	In molars there will be restorations extended to the buccal or palatal smooth surface. At the border of the restorations frequently an opacity can be noticed. In incisors a buccal restoration can be noticed not related to trauma.
Extraction due to MIH	Suspected for extraction due to MIH are: • Opacities or atypical restorations in the other first permanent molars combined with absence of a first permanent molar • The absence of first permanent molars in a sound dentition in combination with demarcated opacities on the incisors is suspected for MIH

Table A.2. Codes and definitions of the combined EAPD diagnostic criteria and the Modified DDE index proposed by Ghanim et al., 2015

Codes and Description of clinical criteria	
0	**No visible enamel defect:** Tooth/surface is apparently free of enamel lesions represented by diffuse opacities, hypoplasia, demarcated hypomineralization and amelogenesis imperfecta.
1	**Enamel defect, not MIH/HSPM:** Quantitative or qualitative defects that are not comply with the characteristic features mentioned in the MIH/HSPM definitions. These defects include the following; 11: **Diffuse opacities:** These defects can have a linear, patchy or patchy confluent distribution with indistinct borders with the surrounding normal enamel exists. Also includes opacities due to fluorosis 12: **Hypoplasia:** Defect can present as pit, groove and areas of partial or total enamel missing with rounded smooth borders adjacent to the intact enamel. 13: **Amelogenesis imperfect:** Includes a range of enamel malformations, genomic in origin, and include variations in thickness (hypoplastic malformation), smoothness and hardness (hypocalcified and hypomatured malformation) or a combination of these. 14: **Other hypomineralization defects:** Only for teeth not included in the MIH/HSPM definition but show hypomineralization defects consistent with the clinical appearance of MIH/HSPM.
2	**Demarcated opacities:** A demarcated defect involving an alteration in the translucency of the enamel, variable in degree from white/creamy to yellow/brown in color. The defective enamel is of normal thickness with a smooth surface and a clearly defined boundary from adjacent, apparently sound, enamel. **21:** White or creamy opacities **and 22:** Yellow or brown opacities
3	**Post-eruptive enamel breakdown (PEB):** Is a defect that indicates loss of initially formed surface enamel subsequent to tooth eruption that it appears clinically as if the enamel has not formed at all. The loss is often associated with a pre-existing demarcated opacity. PEB exists on surfaces traditionally considered at low caries risk (i.e. cuspal ridges and smooth surfaces) and its areas are rough and have uneven margins.
4	**Atypical restorations**: The size and shape of restorations do not conform to the usual picture of plaque related caries. In most cases in posterior teeth there will be restorations extended to the buccal or palatal smooth surfaces. The restorations may have residual affected enamel visible at the margins.
5	**Atypical caries:** The size and form of the caries lesion do not match the present caries distribution in the patient's mouth. The unusual pattern of caries can be further confirmed as associated to MIH/HSPM if signs of MIH/HSPM are seen in other teeth in the same mouth.
6	**Atypical extraction (Missing due to MIH/HSPM):** Suspect when absence of a FPM or SPM in an otherwise sound dentition and associated with opacities, PEB, atypical restorations or atypical caries in at least one of the FPM or SPM. It is unlikely that PIs will be extracted due to MIH.

7	**Defect cannot be scored:** Index tooth with extensive coronal breakdown and where the potential cause of breakdown is impossible to determine.
Codes and description of the defect extent	
	I: Less than 1/3 of the tooth surface involved II: At least 1/3 but less than 2/3 of the tooth surface involved III: At least 2/3 of the tooth surface involved. The total area affected is to be related to the total

Table A.3. Some of the published reports on the perception of Molar Incisor Hypomineralization among oral health professionals around the globe (ordered by the year of publication).

Country/Region	**Population**	**Sample size (%Response rate)**
European Union (Weerheijm and Mejare 2003)	Members of the European Academy of Pediatric Dentistry (EAPD) in 31 countries	45(92%)
Australia and NZ (Crombie, Manton et al. 2008)	Members of the Australian and New Zealand Society of Pediatric Dentistry	130(59%)
Iraq (Ghanim, Morgan et al. 2011)	Academic faculty in the University of Mosul	146(77.7%)
Malaysia (Hussein, Ghanim et al. 2014)	General dental practitioners (GDPs) and dental nurses (DNs) attending a nationwide dental conference in Melaka, Malaysia	131(58%)
Saudi Arabia (Silva, Alhowaish et al. 2016)	Dentists with active membership in the Saudi Dental Society and fourth and fifth year undergraduate dental students at King Saud University	Dentists: 230 (57%), Dental students: 149(67%)
Chile and Australia (Gambetta-Tessini, Marino et al. 2016)	Chilean and Australian GDPs and oral health therapists (OHTs)	232(29 %)
Hong Kong (Gamboa, Lee et al. 2018)	General dental practitioners (GDPs) and pediatric dentists (PDs) practicing in Hong Kong	255(43%)

Table A.4. Description of the ICDAS codes and criteria

ICDAS Code	Description
0	Sound tooth surface: There should be no evidence of caries. Surfaces with developmental defects such as enamel hyperplasia, fluorosis, tooth wear (attrition, abrasion, and erosion), and extrinsic or intrinsic stains will be recorded as sound. The examiner should also score as sound, a surface with multiple stained fissures if such a condition is seen in other pits and fissures
1	First visual change in enamel. When seen wet there is no evidence of any change in color attributable to carious activity, but after prolonged air drying, a carious opacity or discoloration (white or brown lesion) is visible, which is not consistent with the clinical appearance of sound enamel, or when there is a change of color due to caries it is not consistent with the clinical appearance of sound enamel and is limited to the confines of the pit and fissure area (whether seen wet or dry). The appearance of these carious areas is not consistent with that of stained pits and fissures as defined in code 0.
2	Distinct visual change in the enamel. The tooth must be viewed wet. When wet there is a carious opacity (white spot lesion) and/or brown carious discoloration that is wider than the natural fissure/fossa, which is not consistent with the clinical appearance of sound enamel. When wet, there is a white spot lesion and/or brown carious discoloration.
3	Initial localized enamel breakdown without visual signs of dentin involvement. The tooth viewed wet may have a clear carious opacity (white spot lesion) and/or brown carious discoloration that is wider than the natural fissure/fossa, which is not consistent with the clinical appearance of sound enamel. Once dried, there is carious loss of tooth structure at the entrance to, or within the pit or fissure/fossa. This will be seen visually as evidence of demineralization at the entrance to or within the fissure or pit, and although the pit or fissure may appear substantially and unnaturally wider than normal, the dentin is not visible in the walls or base of the cavity/discontinuity.
4	Underlying dark shadow from the dentin with or without enamel breakdown. This lesion appears as a shadow of discolored dentin visible through an apparently intact enamel surface, which may or may not show signs of localized breakdown. The shadow appearance is often seen more easily when the tooth is wet. The darkened area is an intrinsic shadow that may appear gray, blue or brown. The shadow must clearly represent caries that started on the tooth surface being evaluated. If in the opinion of the examiner, the carious lesion started on an adjacent surface and there was no evidence of any caries on the surface being scored, then the surface should be coded “0”.
5	Distinct cavity with visible dentin cavitation in opaque or discolored enamel with exposed dentin. Cavitation in opaque or discolored enamel, exposing the dentin beneath. The tooth viewed wet may have darkening of the dentin visible through the enamel. Once dried, there is visual evidence of loss of tooth structure at the entrance to or within the pit or fissure - frank cavitation. There is visual evidence of demineralization (opaque (white), brown or dark brown walls) at the entrance to or within the pit or fissure and in the examiner's judgment, the dentin is exposed.
6	Extensive distinct cavity with a clearly visible dentine. There is obvious loss of tooth structure, the cavity is both deep and wide, and the dentin is clearly visible on the walls and at the base. An extensive cavity involves at least half of a tooth surface or possibly reaches the pulp.

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CURRICULUM VITAE

Azza Tagelsir Mohamed Ahmed

Education

2014-2020 Doctor of Philosophy (Ph.D.) in Dental Sciences, Indiana University, School of Dentistry, Indianapolis, Indiana, USA

2006-2009 Master of Science in Pediatric Dentistry and Special Care (MSc), Ghent University, School of Dentistry, Ghent, Belgium

2006-2009 Certificate in Pediatric Dentistry, Ghent University, School of Dentistry, Ghent, Belgium

1999-2001 Bachelor of Dental Surgery (BDS), University of Khartoum, School of Dentistry, Khartoum, Sudan

Academic Professional Experience

2020- current Attending Paediatric Dentistry Faculty, Children's Hospital of Michigan, Michigan, USA

2017- 2018 Instructor, Indiana University School of Dentistry, Department of Cariology, Operative Dentistry and Dental Public Health. Indiana, USA

2009-2014 Clinical Assistant Professor, University of Khartoum, Faculty of Dentistry, Department of Orthodontics, Paediatric Dentistry and Preventive Dentistry, Khartoum, Sudan

Academic Honours and Awards

2019 President's Diversity Dissertation Year Fellowship, Indiana University Purdue University Indianapolis (IUPUI), Indiana, USA

2018 IUPUI Elite 50 Graduate and Professional Student Award in Community Service, Indiana University Purdue University Indianapolis (IUPUI), Indiana, USA

2016 Sam H. Jones Community Service Scholarship, Indiana University Purdue University Indianapolis (IUPUI), Indiana, USA

2009 Master of Science Degree with Great Distinction (Magna cum laude), Ghent University, School of Dentistry, Ghent, Belgium

2000- 2001 The University of Khartoum Awards of the Highest achievement in academic performance for the semi-final and final pre-doctoral years, University of Khartoum Faculty of Dentistry, Khartoum, Sudan

Peer-Reviewed Publications

2020 **A. Tagelsir,** Soto-Rojas AE, JA. Dean, GJ. Eckert, EA. Martinez-Mier. Prevalence of Molar Incisor Hypomineralization and Other Enamel Defects in Indiana, USA (Accepted for publication, Journal of American Dental Association, February **2020**).

2018 **A. Tagelsir,** JA. Dean, GJ. Eckert, EA. Martinez-Mier. U.S. pediatric dentists' perception of molar incisor hypomineralization. July-August **2018**, Pediatric dentistry 40(4):272-78.

2018 AI. Elgamri, **A. Tagelsir**, OE. Haj-Siddig, JR. Chin. Infant Oral Mutilation (IOM) related to traditional practices among inner city pre-school children in Sudan. Afri Health Sci. **2018**;18(2): 359-368.

2018 Lippert F, Al Dehailan L, Castiblanco GA, **Tagelsir AA**, Buckley C, Eckert GJ. Enhancing predicted fluoride varnish efficacy and post-treatment compliance by means of calcium-containing gummy bears. J Dent. **2018** Jun; 73:40-44.

2016 **Tagelsir A**, Yassen GH, Gomez GF, Gregory RL. Effect of antimicrobials used in regenerative endodontic procedures on 3-week-old *enterococcus faecalis* biofilm. J Endod. **2016** Feb;42(2):258-62.

2013 **Tagelsir A**, Khogli AE, Nurelhuda NM. Oral health of visually impaired schoolchildren in Khartoum State, Sudan. BMC Oral Health. **2013** Jul 17; 13:33.

2011 **Tagelsir A**, Cauwels R, van Aken S, Vanobbergen J, Martens LC. Dental caries and dental care level (restorative index) in children with diabetes mellitus type1.Int J Paediatr Dent. **2011** Jan;21(1):13-22.

Oral Presentations

2019 Molar Incisor Hypomineralization in the United States: How Much We Know About It. String of Pearls Session, The **72nd** Annual Session of the American Academy of Pediatric Dentistry (AAPD), Chicago, USA

2018 Where Do the U.S. Pediatric Dental Practitioners Stand regarding Molar Incisor Hypomineralization? The **65th** European Organization of Caries Research (ORCA), Copenhagen, Denmark.

2011 Molar Incisor Hypomineralization (MIH): Are We Aware of The Problem? The **7th** Sudanese Dental Conference, Khartoum, Sudan.

2009 Dental caries and dental care level in children with type 1 Diabetes Mellitus. The **22nd** meeting of the International Association of Pediatric Dentistry (IAPD), Munich, Germany.

Poster Presentations

2019 **Tagelsir AA,** Soto-Rojas AE, Dean JA, Eckert GJ, Martinez Mier AE. Prevalence of Molar Hypomineralization in Indiana, USA. The 66th European Organization of Caries Research (ORCA) congress, Cartagena, Colombia

2018 **Tagelsir AA**, Dean JA, Eckert GJ, Martinez Mier AE. Where Do the U.S. Pediatric Dental Practitioners Stand regarding Molar Incisor Hypomineralization? The 65th European Organization of Caries Research (ORCA), Copenhagen, Denmark

2016 **Tagelsir AA,** Lippert F. Effect of Milk with Strontium and Fluoride on Enamel Surface Microhardness. The 24th Annual Research Day of Indiana University School Dentistry, Indiana, USA

2011 **Tagelsir A**, Martens LC, Marks L. Oral findings and dental management of a patient with Progressive familial intrahepatic cholestasis (PFIC): case

report. The 23rd meeting of the International Association of Pediatric Dentistry (IAPD), Athens, Greece

Service

Academic Service

2016 Teaching an intensive graduate level course focused on the contemporary materials and techniques used in pediatric dentistry for postgraduate pediatric dentistry residents. The course provided both hands-on clinical and didactic activities. University of Khartoum Faculty of Dentistry, Department of Orthodontics, Pediatric Dentistry and Preventive Dentistry, Khartoum, Sudan

2010-2014 Member of examiner committee of pre-doctoral and postdoctoral pediatric dentistry courses, University of Khartoum Faculty of Dentistry, Department of Orthodontics, Pediatric Dentistry and Preventive Dentistry, Khartoum, Sudan

2014 External examiner of pre-doctoral pediatric dentistry, National Ribat University, Faculty of Dentistry, Khartoum, Sudan

Community Service

2015-2016 Graduate Service-Learning Assistant Scholar (Sam H. Jones community Service Scholar), Indiana University Purdue University Indianapolis (IUPUI), Indiana, USA

2016 Pediatric dentist volunteer as part of the Indiana University School of Dentistry AAPD student chapter [Kids Club], Indiana University Purdue University Indianapolis (IUPUI), Indiana, USA

2007-2009 Part of the oral health volunteer team of the "Special Olympics Special Smiles" program organized in different cities, Ghent University, School of Dentistry, Ghent, Belgium

Licensing

2019-present Dentist Clinical Academic Limited License, Michigan, USA

2016-2020 Limited Indiana Dental Residency License, Indiana, USA

2010-present Pediatric Dentist Consultant Registration License, Sudan Medical Board, Sudan

Professional Memberships

2019-present International Association for Dental Research (IADR)

2018-present European Organization for Caries Research (ORCA)

2017-present American Academy of Pediatric Dentistry

2015-present American Association for Dental Research-Indiana section (INAADR)

2006-present European Academy of Pediatric Dentistry

2006- present Belgian Society of Pediatric Dentistry