Prevalence and Presentation Patterns of Enamel Hypomineralisation among Paediatric Hospital Dental Patients in Toronto, Canada

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science
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Abstract

Purpose: To determine the prevalence and presentation patterns of molar incisor hypomineralisation (MIH) and hypomineralised second primary molars (HSPM) in the Division of Paediatric Dentistry at The Hospital for Sick Children in Toronto, Canada.

Methods: A cross-sectional study of 429 participants was performed by 9 calibrated examiners. The study used the European Academy of Paediatric Dentists criteria in diagnosing and documenting MIH/HSPM based on clinical presentation, size, and location of defects.

Results: MIH prevalence was 12.4% (n=29) and 5.2% (n=19) for HSPM. Demarcated white opacities were the most common clinical finding of MIH (60%) and HSPM (67%). In both MIH and HSPM, single surface hypomineralised lesions were significantly more common than multi-surface lesions (p<0.0001). Lesion extension, less than 1/3 of a tooth surface was significantly more common than those greater than 1/3 (p=0.0001).

Conclusions: Prevalence of MIH and HSPM from this North American sample were within the range of published studies from other continents.
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Chapter 1

1 Introduction

1.1 Background

Molar incisor hypomineralisation (MIH) is defined as “hypomineralisation of systemic origin of one to four first permanent molars frequently associated with affected incisors” (Weerheijm et al. 2003). In addition, a second term, hypomineralised second primary molars (HSPM) describes a similar finding on second primary molars (Elfrink et al. 2008).

MIH and HSPM have variable clinical presentations within and among patients. Most patients with MIH or HSPM report no symptoms and present with white, yellow or brown opacities on their first permanent molars and/or incisors or second primary molars. The borders of these enamel opacities are generally well demarcated from healthy enamel, reflecting focal areas of enamel hypomineralisation. In severe cases, the hypomineralised enamel undergo post-eruptive breakdown (PEB) from masticatory forces, exposing underlying dentin. Patients with MIH have a higher caries rate than those with no enamel defects (Americano et al. 2017).

Hypomineralised enamel demonstrates disorganised enamel crystals with pronounced interprismatic spaces and have greater protein content than healthy enamel. The enamel porosity and distinct composition seen in MIH teeth were implicated in PEB and less favourable resin restoration outcomes when compared to healthy enamel (Crombie et al. 2013; William et al. 2006b). Inflammatory cells observed in the pulps of MIH teeth correlated with reports of sensitivity to thermal, chemical and physical stimuli in some patients (Rodd et al. 2007). The higher caries experience in patients with MIH compared to those without from a recent systematic review can be explained by the ease of bacterial ingress through the porous enamel (Americano et al. 2017). Rarely, symptoms of sensitivity associated with hypomineralised teeth can also make routine oral hygiene challenging (Oliver et al. 2014).

Jalevik and Klingberg reported that patients with MIH were 10 times more likely to require dental treatment and repeat treatments on their first permanent molars than their unaffected peers at 9 years old (Jalevik, Klingberg 2002). Increased treatment needs were again observed at
follow-up at 18 years of age (Jalevik, Klingberg 2002). Patients with MIH experience more dental anxiety and fear than patients without MIH according to patient and parental reports (Jalevik, Klingberg 2002). Clinicians also experience anxiety in treating patients with MIH due to their lack of confidence and knowledge in providing definitive diagnosis and treatment (Kalkani et al. 2016). In particular, clinicians reported difficulty in obtaining adequate anaesthesia in patients with MIH that might explain these patients’ reports of greater discomfort during dental treatment compared to their peers. Patients with MIH and their families incur significant financial and intangible costs from symptoms, treatments and missed school and work time (Mathu-Muju, Kennedy 2016).

The range of MIH prevalence has been reported as 2.8 to 44% (Hernandez et al. 2016). HSPM prevalence is lower than MIH prevalence, ranging from 0 to 9% (Elfrink et al. 2015). It is challenging to compare prevalence values among studies due to differences in demographic samples and the inconsistent use of standardised diagnostic criteria. Currently, there are no published prevalence values for MIH or HSPM from North American samples.

1.2 Definitions and Diagnosis

During the 5th Congress of the European Academy of Paediatric Dentistry (EAPD) in 2000, four separate investigative groups described similar enamel developmental defects on first permanent molars (Weerheijm et al. 2001). The terminologies used to describe the defects included, “hypomineralised”, “idiopathic enamel hypomineralisation”, “nonfluoride hypomineralisation”, and “cheese molars” (Weerheijm et al. 2001). While the terminologies varied, the clinical descriptions of enamel defects ranged from white and brown demarcated opacities to broken down enamel. Clinicians were familiar with enamel hypomineralisation on first permanent molars but there was no existing diagnostic label that accurately described this phenomenon. To address this deficiency, the European Academy of Pediatric Dentistry (EAPD) dedicated “Mineralisation Defects of Permanent First Molars” as a theme in the following EAPD Congress Meeting in 2002. At this meeting, Weerheijm introduced the term, MIH, to amalgamate a variety of terms that previously existed to describe the same enamel condition. Drs. Weerheijm, Fayle and Walsh also discussed the inadequate published research regarding epidemiologic data, treatment recommendations and prognosis of teeth affected by MIH. In 2003, Weerheijm and others held a meeting to establish criteria for diagnosing MIH, to select clinical cases that best
represented the condition for education purposes and to standardise terminology (Weerheijm et al. 2003). In 2003, Weerheijm published *The Judgment Criteria for MIH*. The intent of this publication was to propose a set of clinical diagnostic criteria to identify MIH as a distinct clinical entity from other known enamel defects such as enamel hypoplasia, fluorosis or amelogenesis imperfecta (Weerheijm et al. 2003). The *Judgement Criteria for MIH* became the standard criteria in diagnosing MIH. Other interchangeable terms found in literature include EAPD Criteria, EAPD Scale or EAPD Guidelines. According to the diagnostic criteria, there are five clinical presentations of MIH: demarcated opacity, post-eruptive breakdown (PEB), atypical caries, atypical restorations and extracted first permanent molar(s) due to MIH (Weerheijm et al. 2003). The opacities are areas of demarcated hypomineralisation. The hypomineralised regions demonstrate altered enamel translucency presenting as white, yellow or brown opacities. The other four presentations listed in the diagnostic criteria are possible clinical outcomes of untreated (PEB and atypical caries) or treated (atypical restorations and extractions) teeth with MIH. The term atypical is used to describe caries and/or restoration that are unlike typical caries presentations or restoration outline forms. Investigators have also applied the EAPD Criteria to the diagnosis of HSPM for second primary molars (Elfrink et al. 2008). Since 2000, the European Archives of Paediatric Dentistry published two special issues on MIH (Weerheijm 2015). The discussions at the 2009 EAPD MIH Interim Symposium culminated in the EAPD MIH Best Practice Clinical Guidelines (Lygidakis et al. 2010). The second special issue on MIH, published in 2015, reflected the knowledge gained about the ultrastructure of the hypomineralised defects, possible genetic predisposition and inclusion of HSPM as part of the MIH spectrum. Uncertainties remain for MIH pathogenesis and treatment protocols. The EAPD criteria for MIH were also updated with the addition of a data collection form with the intent of standardising MIH research (Weerheijm 2015; Ghanim et al. 2015).

### 1.3 Clinical Presentation

MIH and/or HSPM patients have been reported in the literature with variable presentations. By definition, all MIH cases involve at least one hypomineralised first permanent molar. All HSPM cases involve at least one hypomineralised second primary molar. There are numerous possible manifestations of MIH and HSPM because each of the affected first permanent molars, permanent incisors and/or second primary molars can independently have one of the five clinical presentations as described by the *Judgement Criteria for MIH*. Investigators observed differences
in the number of teeth affected and the tooth surface affected (buccal, palatal/lingual, occlusal/incisal) among patients with MIH. Additionally, each hypomineralised defect can independently manifest as demarcated opacities, PEB, atypical restorations, atypical caries or missing due to history of extraction. Patients can concurrently have MIH and HSPM. According to the EAPD Criteria, demarcated opacities greater than 1mm found on first permanent molars, incisors and second primary molars are included for MIH and HSPM diagnoses (Lygidakis et al. 2010). These hypomineralised defects are often located on the occlusal and buccal aspects of the crown. The weak porous enamel can lead to rapid breakdown upon eruption that is associated with sensitivity and rapid caries progression. Some patients with MIH have atypical caries or atypical restorations on their first permanent molars and less commonly, incisors.

All five clinical manifestations of MIH are described in epidemiological studies (Willmott et al. 2008; Lygidakis et al. 2008; Lygidakis et al. 2010). However, investigators failed to demonstrate any consensus on the number of the first permanent molars and/or incisors involved and/or the tooth surface(s) (occlusal, buccal/labial, palatal/lingual) involved (Willmott et al. 2008). Some studies concluded a higher probability of incisor involvement when an increased number of first permanent molars were affected by MIH (Chawla et al. 2008a; Oliver et al. 2014). There is no conclusive association among the number of teeth affected, the surface location of the defect and the severity of MIH. Most studies reported no predilection for sex or social economic status (Taylor 2017).

Investigations on the presentation patterns of HSPM are scarce. The EAPD criteria are applied to the diagnosis of hypomineralised second primary molars. In one study, stainless steel crowns were considered to be atypical restorations in an otherwise unrestored dentition (Elfrink et al. 2008). The same author concluded in another study that patients with HSPM were four times more likely to develop MIH than patients without HSPM and thus, HSPM could be a prognostic for MIH (Elfrink et al. 2012). Ghanim and colleagues did not find a significant relationship between the presence of HSPM and MIH in their study but attributed this to a small sample size (Ghanim et al. 2013b).

Mathu-Muju and Wright categorized MIH severity based on different clinical manifestations of MIH (Mathu-Muju, Wright 2006). The authors developed the following classification: mild MIH was asymptomatic, demarcated opacities in non-stress-bearing areas of first permanent molars;
moderate MIH had presence of atypical restorations, opacities on stress-bearing areas not associated with PEB, and if present, PEB was limited to one or two surfaces without cuspal involvement; severe MIH had PEB or loss of enamel during eruption, associated dental sensitivity, defective restorations, and possible pulpal complications.

Similar demarcated opacities were also reported on second primary molars, the tips of permanent canines, premolars, permanent second molars and incisors without molar involvement but it was unclear if they each represented a distinct condition or belonged on a spectrum with MIH (Lygidakis et al. 2010). The EAPD MIH Policy Statement acknowledged this uncertainty but proposed that for the time being, the term MIH should still be used to describe hypomineralisation found exclusively on first permanent molars and/or incisors. It has been recommended that future research on enamel hypomineralisation include assessment and recording of enamel defects on all clinically present teeth (Lygidakis et al. 2010; Lacruz 2017; Elfrink et al. 2015).

1.4 Amelogenesis

Enamel consists of 96% hydroxyapatite crystals and 4% organic materials and water (Bartlett 2013). The hydroxyapatite crystals are bundled into enamel prisms or rods that contain 60% calcium by weight. Enamel is the most highly calcified structure found in vertebrates and the only epithelial-derived tissue that undergoes nonpathological mineralisation in mammals (Lacruz 2017). The high mineral content found in enamel renders the surface of the tooth resistant to wear and demineralisation. Unlike other mineralised tissues of the body such as bone, cartilage, cementum and dentin, enamel is of epithelial origin, non-collagenous, and does not undergo remodeling (Lacruz 2017). Bone mineralises as it is formed and continuously remolds (Lacruz 2017). Enamel mineralises in two phases, secretion and maturation. Once enamel is formed, it does not regenerate or remodel. The enamel thickness is greatest at cusp tip of molars, approximately 2.5mm, and tapers to a feather edge at the cementoenamel junction.

Ameloblasts guide a well-orchestrated and genetically involved process of amelogenesis. The most common ions in enamel crystals are calcium and phosphate but the enamel crystals also have varying compositions of bicarbonate, chloride, potassium, sodium and magnesium ions during different stages of amelogenesis (Lacruz 2017).
Ameloblasts are differentiated from the inner enamel epithelium (IEE) of the enamel organ (See Figure 1). Other components of the enamel organ include the outer enamel epithelium (OEE), stellate reticulum and stratum intermedium. The OEE is a single layer of cells that cover the enamel organ and is continuous with the IEE. The early ameloblasts of IEE are attached to stratum intermedium on the basal/proximal end and to the basement membrane or future dentoenamel junction (DEJ) on the apical end (Bartlett 2013). The stratum intermedium cells are important for transporting ions to and from ameloblasts. The stellate reticulum cells are located between the stratum intermedium and OEE. Ameloblasts form a single cell layer that covers the developing enamel. Amelogenesis takes place in three phases: differentiation, secretion (presecretory and secretory), and maturation.

Fig 1: A: Ameloblasts (am) differentiate from IEE at the cusp tip (1) on dentin surface towards the CEJ (2). Od = odontoblasts, p = pulp, si = stratum intermedium, sr = stellate reticulum, ek = enamel knot. B: Direction of ameloblasts differentiation from cusp tip (1) to where the IEE fuses with OEE (2). Thickness of enamel (3) is established during secretory phase. Ameloblasts at the cusp tip (4) start the transition from secretion to maturation phase. This transition occurs in a similar wave like pattern from cusp tip (5) to cervical margin (6). At this stage, the morphology of the enamel is established as amelogenesis completes secretion phase and enters maturation stage. Source: Simmer (Simmer et al. 2010)

During each phase of amelogenesis, ameloblasts undergo morphological changes, inferring a possible role of cell form during amelogenesis. The morphological changes seen during the ameloblast’s lifespan are divided into 7 stages: induction, differentiation to ameloblasts,
presecretory ameloblasts with villi processes, secretory ameloblasts with Tomes’ processes, secretory ameloblasts with retruded Tomes’ processes, transition ameloblasts, maturation ameloblasts (ruffle-ended, RE and smooth ended, SE) (See Figure 2) (Varga et al. 2015). Ameloblasts are genetically programmed to secrete various proteins during their life cycle at specific times and quantities. The enamel proteins self-assemble to form an enamel extracellular organic matrix that will become a scaffold that controls the initiation, growth rate and location of enamel crystals. During the maturation phase, almost the entire organic protein matrix is degraded, removed and replaced by enamel crystals (Lacruz et al. 2017).

There are various environmental influences and genetic alterations that can potentially disrupt enamel development. The enamel manifestations depend on the type, timing, intensity and duration of the disturbance. In general, insults during the secretory phase are associated with quantitative defects of enamel or enamel hypoplasia. Insults during the maturation phase are associated with qualitative defects or enamel hypomineralisation. Stressors of short duration such as fever often result in localized defects and in contrast, chronic stressors are more likely to result in generalised defects (Lacruz et al. 2017). The details on how ameloblast functions are changed in cases of MIH and HSPM are uncertain but it is hypothesized that it occurs during maturation phase.

Fig 2: Ameloblast morphological changes during amelogenesis. Induction of IEE (1), differentiation from IEE to ameloblasts (2), pre-secretory (3), secretory with Tomes’ processes (4), secretory with retruded Tomes’ processes (5), transition (6), rough-ended maturation and smooth-ended maturation (7) Source: Hu et al (Hu et al. 2007)
Understanding of amelogenesis comes from laboratory studies that examined the structural properties of ameloblasts via microscopy, immunohistochemistry and patch clamp studies (Farah et al. 2010a). The functional properties of ameloblasts were elucidated in animal models. Mouse models have been instrumental in defining the role of enamel proteins. Mouse models are advantageous because the mouse genome is known. Additionally, mice mature quickly, their incisors grow continuously, and investigators are familiar with the structural and biochemical differences between mice and human incisors. Investigators use transgenic models with either knocked-in or knocked-out genes to assess the effects of over-expression or under-expression of protein(s). By comparing the enamel phenotypes of the transgenic mice with wildtypes, the functions of selected proteins are deduced. Mice deficient in AMEL (amelogenin), AMBN (ameloblastin), ENAM (enamelin), AMTN (amelotin), MMP20 (matrix metalloproteinase-20) and KLK4 (kallikrein related peptidase 4) demonstrate varying degrees of hypoplastic, hypocalcified, or hypomatured enamel (Nakayama et al. 2015). Ultimately a better understanding of amelogenesis may help to clarify the etiology of enamel defects including MIH and/or HSPM.

The secretion phase of amelogenesis starts with the deposition of predentin by odontoblasts. The secretion phase is a two-stage process that starts with the creation of a minimally mineralised matrix and is followed by the remodeling of the matrix to increase its mineral content. During the secretion phase, ameloblasts exhibit reverse nuclear polarity and become columnar in shape with more extensive rough endoplasmic reticulum and an increased number of mitochondria and Golgi bodies (Bartlett 2013; Varga et al. 2015). These organelles support the active transport process of proteins that takes place during the secretion phase. The initial enamel crystals, referred to as ribbons, mineralise between dentin crystals and form a mineralisation front, where the secreted ameloblasts proteins contact the newly mineralised dentin (Bartlett 2013). This interface becomes the dentoenamel junction (DEJ). The first layer of mineralised enamel is aprismatic (Bartlett 2013). Secretion phase ameloblasts begin to exhibit Tomes’ processes that are conical extensions at the distal cell process that are important in the organization of enamel crystals. Tomes’ processes are the sites for protein secretion from the intracellular space of the ameloblasts to the immediate extracellular space (Bartlett 2013). As the Tomes’ processes develop, organic matrix surrounds the hexagonally-shaped enamel crystals. The ameloblasts no longer replicate and instead, produce enamel matrix proteins, three structural molecules and one
proteinase: MMP-20 (Bartlett 2013). The structural proteins consist mostly of amelogenin and lesser amounts of ameloblastin and enamelin (Varga et al. 2015). After the amelogenins are secreted by the ameloblasts into the extracellular space, the amelogenins aggregate to form nanospheres (Varga et al. 2015). The nanospheres form sheets that separate and orient the newly formed enamel crystallites. A distinguishing feature of enamel mineralisation as opposed to conventional mineralisation of dentin or bone, is that one protein, amelogenin, assumes the role of both the scaffold and the center of crystal growth (Varga et al. 2015). Dentin and bone mineralisation require a collagen scaffold, followed by mineralisation. Calcium and phosphate ions are actively transported from the basolateral surface to the apical surface of ameloblasts to start mineralisation. At the end of the secretory phase, the enamel has obtained its final thickness but with only 30% of its final mineral content (Varga et al. 2015). The mineralised content is configured into parallel crystal ribbons with sheets of amelogenin sandwiched in between (Varga et al. 2015).

During the maturation phase, ameloblasts retract their Tomes’ processes and change their morphology, alternating between RE and SE phenotypes. The organic matrix previously secreted by the Tomes’ processes is degraded and removed. Consequently, the enamel crystals increase in length, thickness and mineralisation content by the increase in calcium and phosphate ions. Radiolabelled calcium and phosphate ion studies confirmed this finding during the maturation stage (Lacruz 2017). At the end of the maturation phase, enamel reaches its final mineralisation content of 96% by weight. Remodeling in the distal portion of the ameloblasts cell is paired with the relocation of tight junctions from distal position in RE to apical position in SE ameloblasts. The two alternating morphologies enable ameloblasts to have two functions (Josephsen et al. 2010). First, the maturing RE ameloblasts secrete calcium and phosphate ions and decrease the pH of the enamel crystal. Second, the ameloblasts reabsorb and degrade the amelogenin proteins that are cleaved by MMP-20 and KLK-4 (Bronckers 2017). The maturation stage ameloblasts secrete KLK-4. Mice without MMP20 via gene-knockout, developed hypoplastic and hypomineralised enamel (Bartlett 2013). Mice with knocked-in KLK-4 gene that demonstrated spatiotemporal over expression of KLK-4 had hypomineralised enamel, emphasizing the importance of not only protein quantity but also timing of expression. Earlier expression of KLK-4 resulted in premature removal of amelogenin before mineralisation has sufficiently matured. Enamel matrix proteins are necessary for enamel formation but not part of the final
mature enamel product as evident by the replacement of amelogenin nanospheres by growing enamel crystal.

Ameloblasts are responsible for creating and controlling a local environment of specific pH and osmolarity that regulates the formation and mineralisation of enamel crystals. This compartmentalised space, the enamel layer, is between the mineralised dentin and the ameloblasts layer and contains the DEJ (Bronckers 2017). The ameloblastic layer recedes from the DEJ as new enamel is formed. Adjacent ameloblasts share tight junctions at the proximal and distal ends of their plasma membrane. The tight junctions regulate the paracellular movement of molecules from the stratum intermedium to the enamel layer. Additionally, the intercellular permeability of ameloblasts depends on the different protein components that make up the tight junction complex (Bronckers 2017). Ameloblasts in the secretory phase had leakier tight junctions, thus more permeable to the surrounding environment of enamel layer than ameloblast membranes in maturation phase (Bronckers 2017).

Ameloblasts regulate transcellular movement of ions (See Figure 3). Ion transporters located on ameloblasts were variably functional during amelogensis. The type and localisation of these channels were achieved via patch clamp technique (Bartlett 2013). The patch clamp technique is the gold standard for studying ion channel activity (Brown, Greenberg 2016). The function of the ion channels in ameloblasts was elucidated via transgenic mouse models. To date, 19 genes were identified and/or localized for Ca\(^{2+}\), HPO\(_{4}^{2-}\), Mg\(^{2+}\), Cl\(^{-}\), HCO\(_{3}^{-}\) and H\(^{+}\) in mouse ameloblasts (Bronckers 2017). The latter three transporters were responsible for acid-base regulation, specifically the neutralisation of protons released during mineralisation. Hypomineralised enamel was observed in transgenic mouse models with knocked-out genes responsible for sodium potassium calcium exchanger 4 (NCKX4), cyclin M4 in exchanging magnesium for sodium (Cnnm4), magnesium channel, anion exchanger 2 (AE2), sodium bicarbonate exchanger 1 (NBCE1), and cystic fibrosis transmembrane conductance regulator (CFTR) in channeling chloride and bicarbonate ions (Bronckers 2017).
Fig 3: Schematic showing ion channels and transporters in a ruffled ended mature ameloblast. At the basal pole, bicarbonate ion is transported by Na\(^+\)/HCO\(_3\)\(^-\) cotransporter (NBCe1). Carbonic anhydrase (CA2) also produces intracellular HCO\(_3\)\(^-\). Anion exchanger (AE2) extrudes the excess HCO\(_3\)\(^-\) at the lateral pole. SLC26a extrudes excess HCO\(_3\)\(^-\) at the apical pole. Na\(^+\)/H\(^+\) exchanger (NHe1) removes H\(^+\) from CA2 activity and other cytosolic sources. AE2, SLC26a and CFTR regulate Cl\(^-\) movement. Ca\(^{2+}\) activated release activated Ca\(^{2+}\) channel controls influx of Ca\(^{2+}\). Sarco-endoplasmic reticulum SERCA2a pump, Na\(^+\)/Ca\(^{2+}\)/K\(^+\) exchanger (NCKX4), CA\(^{2+}\)-ATPases (PMCA), Na\(^+\)/Ca\(^{2+}\) exchanger (NCX) control Ca\(^{2+}\) efflux. At the distal pole, Na\(^+\)/PO\(_4\)\(^{3-}\) regulates phosphate movement. Transient receptor potential cation channel subfamily M member 7 (TRMP7) moves Mg\(^{2+}\) into the cell and cyclin and CBS domain divalent mental cation transporter mediator 4 (CNN4) removes Mg\(^{2+}\) from the cell. N=nucleus, ER=endoplastic reticulum, GJ=gap junction. Source: Lacruz (Lacruz 2017)

To further demonstrate the complexity of amelogenesis, investigators show a coupling of form and function in the role of pH modulation during enamel maturation. RE ameloblasts have greater capacity to transport ions between the ameloblast and extracellular space and underwent endocytosis of enamel matrix protein debris. SE ameloblasts are designed for intercellular communication (Lacruz et al. 2017). The cyclic transformations of RE and SE ameloblasts correspond to the altering of pH between 6.2 and 7.2. The exact mechanism of this cyclic change is unknown but is associated with cyclic changes in pH, ion concentration levels, mineral
content, transporter expression and overall increase in crystalline structure. Cyclic modulation was closely paired with buffering because low levels of Cl− impaired cyclic modulation and hypomineralised enamel was observed (Bronckers 2017). Ameloblasts were more metabolically active during the RE state compared to the SE state. Decrease in pH during mineralisation was associated with change in calcium gradient across the ameloblasts cell membrane. Increased ameloblast intracellular calcium levels and acidity were thought to trigger RE to SE transformation. SE state was considered to be a recovery state from the periods of high acidity. It is hypothesised that by cycling between RE and SE, ameloblasts endured less stress to ensure adequate lifespan to allow for complete mineralisation. During the secretion phase, at pH 7.2, the immature enamel crystal contained substantial amounts of magnesium and carbonate, which weakened the structural integrity. A decrease in the pH in ameloblasts selectively dissolved these impurities, followed by an increase in pH that allowed for the recrystallization of the enamel (Bronckers 2017). The cyclic pH change, responsible for the mineralisation of enamel, was reflected in the gradual increase in calcium:phosphate ratio in enamel. Mouse with knocked out anion exchanger 2 (Ae2) and CFTR had higher Ca:P ratio compared to their wild types, due to increased acidity. Modulation between the RE and SE state buffered and interrupted the acidic condition associated with enamel mineralisation. This ultimately resulted in adequate enamel mineralisation (Bronckers 2017).

1.5 Aetiology

The aetiology of MIH is still unclear and likely multifactorial (Crombie et al. 2009; Silva et al. 2016). The term MIH is adopted because it carries no aetiological connotation (Weerheijm et al. 2003). Some observational studies attempted to determine aetiological factors for the development of MIH. Investigators suggested disturbances during the maturation stage of amelogenesis to be the putative cause of MIH. In first permanent molars, permanent incisors and second primary molars, this susceptible period of enamel development corresponds to the perinatal period (Elfrink et al. 2012). Although perinatal illnesses or events had been implicated in the development of MIH, no cause was definitively identified. Suggestions of causes of enamel hypomineralisation such as antibiotics or environmental pollutants as primary factors for MIH are refuted by finding of a 7th-11th century human specimen with teeth affected MIH and HSPM (Garot et al. 2017). In the most recent MIH review publication, only 28 out of 254 identified MIH and/or HSPM aetiological studies met the inclusion criteria of being a cohort
and/or case-control study design with a measureable outcome, defined as presence or absence of MIH and/or HSPM (Silva et al. 2016). There was little evidence of an association between the most frequently investigated prenatal factors, including maternal smoking, maternal illness and maternal medication use and the development of MIH. All six studies examining maternal smoking failed to yield a significant association. Four out of the 10 studies examining maternal illness during pregnancy reported significant results. However, Sonmez and colleagues’ study accounted for confounding variables via multiple logistic regression, concluded that the association was insignificant after regression analysis (Sonmez et al. 2013). One study showed that maternal stress during pregnancy was associated with higher odds of developing MIH than mothers who reported lower stress (OR 3.24, CI 1.33-7.88) (Ghanim et al. 2013a). Mothers who reported greater stress also experienced more illnesses during pregnancy. Subjectivity of measures of stress and participant recall bias were factors that weakened an association between pregnancy stress and development of MIH. Minimal support for an association between perinatal factors including prematurity, low birth weight, caesarean delivery and birth complications and MIH was reported. Only 3 out of 14 studies reported an association between prematurity and MIH (Silva et al. 2016). Studies examining early childhood illnesses (under age of 4 years) and MIH showed a bias towards respiratory illnesses including pneumonia and asthma. After adjusting for confounding values, two studies did not find any significant associations between asthma and MIH (Kuhnisch et al. 2014; Sonmez et al. 2013). This conflicted with conclusions drawn by other retrospective studies that did not perform multiple regression analysis (Silva et al. 2016). Interestingly, Kuhnisch and colleagues found a statistically significant relationship (OR=2.48) between asthma and the development of MIH with incisor involvement but not between asthma and MIH without molar involvement (Kuhnisch et al. 2014). The effect of amoxicillin on development of MIH was unknown as both retrospective and prospective investigations yielded conflicting results. Similarly, no conclusions could be made for asthma medication and MIH (Silva et al. 2016). Other post-natal factors that were implicated in the aetiology of MIH but lacked evidence included measles, chicken pox, tonsillitis, adenoiditis, and otitis media (Silva et al. 2016). Recent studies refuted previous claims on the association between dioxin in breast milk and MIH (Laisi et al. 2008). The majority of the existing MIH and/or HSPM etiological studies depended on retrospective recall by the parent that was subjected to recall bias. Even if the parental reports were accurate, and verified by medical records, it was not possible to conclude associative relationships without ruling out confounding
factors. Proposed etiological factors such as perinatal disturbances were difficult to quantify and/or measure objectively and to be considered in isolation. For example, evidence for the association between amoxicillin and development of MIH was inconclusive or weak. The development of MIH could be attributed to participants’ underlying medical condition or the use of amoxicillin. If the latter were true, the relationship between MIH and amoxicillin can be dose dependent. At this point, no conclusions could be drawn about any suggested perinatal events and the subsequent development of MIH and/or HSPM.

Currently, the genetic loci that corresponded to proteins with a wide range of function including enzymes, regulatory proteins, transmembrane proteins, structural proteins, growth factors, and protein folding cofactors were mapped in 71 conditions with enamel manifestations (Wright et al. 2015). Hypoplastic enamel was more common than hypomineralised enamel. The presentation pattern of MIH, confined to first permanent molars and incisors, was traditionally explained by a localised disturbance during enamel maturation as opposed to an underlying genetic predisposition. Enamel defects of systemic origin, such as amelogenesis imperfecta or fluorosis, resulted in a generalised presentation pattern. Jermeias and colleagues were the first group to evaluate a possible association between MIH and variations in genes responsible for enamel formation, including ameloblastin (AMBN), amelogenin (AMELX), enamelin (ENAM), tuftelin (TUFT1) and tufelin-interacting protein 11 (TFIP11) (Jeremias et al. 2013). AMELX, gene responsible for amelogenin production during secretion phase, was not associated with MIH. Associations between genes and development of MIH suggested an epigenetic role, an interaction between genes and environment. Specifically, genes responsible for enamel proteins were affected by epigenetics during the maturation time of first permanent molars and incisors.

1.6 Properties

Investigators observed different structural and chemical properties between hypomineralised enamel and healthy enamel from laboratory studies. MIH defects were distinguished from carious lesions by their location on a tooth, their shape and colour (Farah et al. 2010c). A distinct boundary separates normal from hypomineralised enamel under the microscope. Two common methods to study the mineral density in teeth affected by MIH are X-ray microtomography (XMT) and nanoindentation testing (Farah et al. 2010a). XMT is similar to a miniaturized version of computerised tomography (CT) and can be used to study the microstructure of enamel.
and its properties including mineral density. The nanoindentation testing method is used to measure hardness and elastic modulus of enamel specimens.

Under the electron microscope, transverse sections of hypomineralised molars show that hypomineralised lesions are confined to enamel. MIH is commonly described as a “qualitative defects of enamel”, suggesting normal enamel quantity but abnormal enamel quality (William et al. 2006b; Crombie et al. 2009; Weerheijm et al. 2001). The thickness of enamel in MIH affected teeth is the same as in healthy teeth. Thus, it is hypothesized that whatever insult affected a localised area of a developing tooth occurred after the enamel secretion phase and during the maturation phase of amelogenesis (Crombie et al. 2009). The term “hypoplastic enamel” is inaccurate in describing MIH affected teeth because there is no missing enamel. Any loss of enamel structure associated with MIH occurred after the eruption of the tooth (Farah et al. 2010c).

Hypomineralised areas are randomly distributed through the surface of molars with the cervical regions being less affected (Elfrink et al. 2013). In healthy teeth, mineral density increases from DEJ to enamel surface. In hypomineralised teeth, mineral density decreases from DEJ to enamel surface (see Figure 4). The surface layer of a hypomineralised defect contains higher mineral content than the subsurface enamel but lower than that found on the surface layer of healthy enamel (Crombie et al. 2013). On average, MIH enamel has 19% lower mineral density than sound enamel. The lower mineral density is comparable to white spot lesions associated with caries that had up to 26% lower mineral content than sound enamel (Cochrane et al. 2012). Additionally, hypomineralised areas have lower calcium and phosphate content than adjacent healthy enamel (Jalevik et al. 2001).
Secondary ion mass spectrometry indicates higher carbon content in hypomineralised enamel compared to healthy enamel (Jalevik et al. 2001). Mahoney and colleagues suggested that the high resistance of MIH affected enamel to acid etching was due to higher organic content in MIH enamel than healthy enamel (Mahoney et al. 2004). Farah and colleagues hypothesized differences in organic content between MIH affected teeth and healthy teeth based on the finding that MIH enamel showed increased laser-induced fluorescence compared to healthy enamel. They proposed that this increase in fluorescence was due to a greater quantity of organic fluorophores as opposed to decrease in calcium (Farah et al. 2010b). A sample of first permanent molars affected by MIH was classified by colour (white, yellow, brown). The enamel was dissolved by 20% trichloracetic acid to extract dental proteins (Farah et al. 2010b). Brown enamel showed 15-21 times higher protein content than normal enamel. Yellow enamel showed eight times higher protein content than normal enamel. These findings were similar to increased protein content found in select amelogenesis imperfect types (Farah et al. 2010b). Serum albumin, type I collagen and antitrypsin were found in all MIH and healthy enamel samples. Yellow and brown enamel samples showed higher levels of serum albumin and antitrypsin, and presence of serum antithrombin compared to opaque/white in healthy subgroups (Farah et al. 2010b).
2010b). Albumin bound to apatite crystals inhibited crystal growth. Serine proteinase inhibitors, alpha-1-antitrypsin and antithrombin inhibited protease, KLK-4. KLK-4 was necessary for the degradation of enamel proteins to allow for full mineralisation of enamel. Thus, decreased KLK-4 levels could result in elevated protein content and reduced mineral content in enamel as evident in MIH samples (Farah et al. 2010b). Mangum and colleagues found three to fifteen fold elevation in protein content in hypomineralised molars than healthy molars but approximately normal level of amelogenins (Mangum et al. 2010). The excess protein was hypothesized to be from blood during pre-eruptive period and oral fluid during post-eruptive period. Clinically, higher protein explains weaker mechanical structure in hypomineralised molars compared to healthy molars. Hypomaturation defects contain also higher levels of amelogenin and thus, this is a distinguishing characteristic between hypomineralisation and hypomaturation (Mangum et al. 2010).

Hypomineralised enamel contains fewer organized enamel prisms with increased interprismatic space compared to sound enamel as evident from scanning electron microscope (SEM). Consequently, hypomineralised enamel is more porous (Fagrell et al. 2010). A well-defined border between normal and disorganised enamel rods infers that function is preserved in ameloblasts adjacent to impaired ones. This lends support for the hypothesis that amelogenesis is affected at a specific stage, leading to hypomineralised teeth (Jalevik et al. 2005). SEM imaging shows that even on intact enamel surfaces of MIH affected teeth, the ultrastructure of surface enamel exhibits more depressions, porosities and abrasions (See Figure 5) (Crombie et al. 2013). The porous enamel is permeable to bacterial ingress. Ground sections of intact enamel surfaces show bacterial ingress and chronic inflammatory reactions in the dental pulp. Surfaces of hypomineralised enamel with PEB are irregular and have varied presentation from large porosities to disorganised crystals (Crombie et al. 2013). Demarcated opacities found on hypomineralised enamel have different ultrastructure than white spots associated with caries, some amelogenesis imperfect variants and fluorosis. Demineralisation associated with caries presents as a wedge shape compared to hypomineralised white opacities that extends through the entire enamel thickness. In hypocalcified AI, the interprismatic spaces resemble healthy enamel and not the enamel of hypomineralised teeth that have increased interprismatic space (Jalevik et al. 2005).
Fig 5: The red oval on the top left photograph indicates the areas examined by SEM. Top row micrograph photos are from intact enamel surfaces (surface abrasions, porosities) and the bottom row photos are from PEB surfaces (disorganized enamel crystal structure). Source: Crombie et al. (Crombie et al. 2013)

Some of the observed physical and chemical properties of hypomineralised enamel may explain the signs and symptoms of MIH including differences in colour, PEB, patient’s experience of sensitivity and propensity for caries development. Darker enamel discolouration was strongly correlated with reduced mineral density in MIH affected teeth ($r=0.88$) (Farah et al. 2010a). Hypomineralised enamel in MIH has reduced hardness and modulus of elasticity that may explain PEB (Crombie et al. 2013).

Only one study investigated the mineral density of intact hypomineralised enamel on primary second molars and compared the findings to sound enamel of carious primary molars (Elfrink et al. 2013). The investigators used the same microCT approach for measuring mineral density in the primary molars as other investigators did in hypomineralised permanent molars (Elfrink et al. 2013). Yellow and brown coloured opacities had 20-22% lower mineral density compared to the controls but no difference was found between white coloured opacities and controls (Elfrink et al. 2013). Unlike permanent molars, only cavitated carious lesions on primary teeth had lower mineral density (31% of normal) compared to healthy primary enamel (Elfrink et al. 2016b). Thus, demarcated white opacities on primary molars share similar physical properties to healthy enamel. Similar conclusion cannot be applied to yellow opacities. Findings from laboratory
studies are based on a limited number of specimens because of the variability in hypomineralised lesions.

1.7 Epidemiology

MIH prevalence has been reported to be 2.8 to 44 percent (Hernandez et al. 2016). Excluding outlier studies (>40%), the prevalence of MIH globally ranges from 5 to 20 percent (See Figure 6) (Elfrink et al. 2015). The average prevalence values is 8.3% in Africa, 12.9% in Asia, 16.2% in Europe, 24.92% in Oceania, and 30% in South America (Hernandez et al. 2016). There is an over-representation of studies from Europe and no studies from North America. In a systematic review of epidemiological studies on MIH from 1987 to 2014, 37 studies met the inclusion criteria. To be included in the review, the authors of the primary papers needed to specify the diagnostic criteria used to diagnose MIH, to conduct a calibration exercise for the examiner(s), and to indicate the sample size and age of the participants (Hernandez et al. 2016). An earlier review with less strict inclusion criteria on MIH and/or HSPM prevalence studies was conducted in 2014. After the initial finding of 1078 studies, only 60 studies (52 MIH, 5 HSPM, 3 both) were included (Elfrink et al. 2015). The investigators of both reviews concluded that differences in research protocol made interpretations among studies challenging (Elfrink et al. 2015; Silva et al. 2016).
Fig 6: Scatter plot shows an increasing number of MIH epidemiological studies from 1987 to 2017. There is no prevalence data reported from North America. Source: Elfrink et al. and Schwendicke et al. (Elfrink et al. 2015; Schwendicke et al. 2018)

The wide range of reported MIH prevalence can be explained by inherent differences in sample populations and/or inconsistencies in research methodologies. Previous studies applied different criteria to define MIH that complicated the comparison of prevalence findings. Only 14 out of the 37 studies included in a review adopted the recommended EAPD criteria and the remaining 13 studies used Koch et al., Alaluusua and Modified Developmental Defects of Enamel Index (mDDE) scales (Hernandez et al. 2016). According to the EAPD criteria, MIH examination should be conducted on clean and moistened teeth at the optimal patient age of 8 years-old (Weerheijm et al. 2003). Strict compliance with the EAPD criteria was poor. Among the studies that used the EAPD criteria, some investigators made modifications to the criteria (Elfrink et al. 2015). Investigators had different study designs such as inclusion/exclusion criteria and
examination protocols. Other examples of study discrepancies include variations in examination conditions (a controlled examination setting such as a dental office with examination light versus a school setting without additional light source), minimum lesion size (1 mm versus 2 mm), age cohorts (5-17 years-old), and exclusion of patients with existing enamel conditions, presence of orthodontic appliances and/or co-morbid medical conditions. Most studies only required data collection for clinically present first permanent molars and incisors as opposed to the entire dentition (Hernandez et al. 2016). The EAPD recommends examination of all teeth for hypomineralisation (Ghanim et al. 2015). By examining only the first permanent molars and incisors, examiners may be biased towards MIH positive findings.

The reported prevalence range of HSPM from eight studies is 0 to 21.8 percent (Elfrink et al. 2015). Kar et al. found no cases of HSPM in their study in India and they were the only group to not use the EAPD scale and had the youngest sample, 3-5 year-olds (Kar et al. 2014). The upper extreme of 21.8% of HSPM prevalence was concluded from the study with the smallest sample size, n=62, compared to the other 7 studies for which sample size ranged from 386-6690 subjects (Elfrink et al. 2009). The studies applied the Judgement Criteria for MIH (EAPD scale) to second primary molars and one group made diagnosis based on intra-oral photographs. According to Elfrink et al, there was a high sensitivity (72.3%) and specificity (92.8%) for scoring HSPM on intra-oral photographs and thus, photographs could be a valid substitute for clinical exam if the same technical conditions were met (Elfrink et al. 2009). Other distinctions in the HSPM epidemiological studies include differences in selected samples (preselected with medical comorbidities or random from school samples), examination conditions (dental chair, school environment, intra-oral photographs) and ages (5-10 year-olds). The considerable variability in criteria for designing and conducting MIH and HPSM prevalence studies led to a wide range of reported values. Currently, there are no reported prevalences for MIH and/or HSPM in North America.

1.8 Measurement Tools

Multiple scales have been used to collect epidemiological data on enamel defects. In the past 50 years, two categories of indices emerged: fluorosis indices and descriptive indices. Descriptive indices included Index of Al-Alousi et al; Developmental Defects of Enamel Index (DDE), modified Developmental Defects of Enamel Index (mDDE) and Enamel Defects Index (EDI).
Before 2003, the majority of MIH investigations used these descriptive scales to study hypomineralisation prevalence but this was problematic as MIH was not defined. The mDDE scale scored lesion outline (diffuse, demarcated), lesion colour (white, yellow) and lesion extension (See Figure 7). There were major drawbacks in using mDDE for scoring MIH and/or HSPM. Firstly, PEB was not accounted for in the mDDE and the closest, but inaccurate, score was hypoplasia. Secondly, there were no scores available for atypical caries, atypical restorations and/or extractions due to MIH. Lastly, completing the mDDE was time consuming and thus, not a practical tool to use in large epidemiological studies. Prevalence studies that used the mDDE were not specific for MIH and the results could be potentially biased by the presentation of other development defects of enamel in the participant sample.

<table>
<thead>
<tr>
<th>A. mDDE index for use in general purpose epidemiological studies</th>
<th>B. mDDE index for use in screening surveys</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Condition</strong></td>
<td><strong>Condition</strong></td>
</tr>
<tr>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Demarcated opacities</td>
<td>Demarcated opacities</td>
</tr>
<tr>
<td>White/cream</td>
<td>0</td>
</tr>
<tr>
<td>Yellow/brown</td>
<td>1</td>
</tr>
<tr>
<td>Diffuse opacities</td>
<td>Diffuse opacities</td>
</tr>
<tr>
<td>Diffuse lines</td>
<td>2</td>
</tr>
<tr>
<td>Diffuse patchy</td>
<td>3</td>
</tr>
<tr>
<td>Diffuse confluent</td>
<td>Diffuse opacities</td>
</tr>
<tr>
<td>Confluent/patchy + staining + loss of enamel</td>
<td>2</td>
</tr>
<tr>
<td>Hypoplasia</td>
<td>Hypoplasia</td>
</tr>
<tr>
<td>Pits</td>
<td>3</td>
</tr>
<tr>
<td>Missing enamel</td>
<td>Any other defects</td>
</tr>
<tr>
<td>Any other defects</td>
<td>4</td>
</tr>
<tr>
<td>Extent of defect</td>
<td>Any other defects</td>
</tr>
<tr>
<td>Normal</td>
<td>0</td>
</tr>
<tr>
<td>&lt;1/3</td>
<td>1</td>
</tr>
<tr>
<td>at least 1/3 &lt; 2/3</td>
<td>2</td>
</tr>
<tr>
<td>at least 2/3</td>
<td>3</td>
</tr>
</tbody>
</table>

Fig 7: An extended mDDE scale used for epidemiological studies (A) and a concise version for screening surveys (B). Both versions were insufficient in describing the array of clinical presentations found in MIH/HSPM including PEB, atypical caries, atypical restorations and extractions. Hypoplastic enamel (pits/missing enamel) is different from hypomineralised enamel.
that underwent PEB. Source: Adapted from Clarkson and O’Mullane (Clarkson, O’Mullane 1989)

The ideal instrument to determine presence or absence of MIH should be easy to use. The need for standardized MIH data collection in epidemiological studies was emphasized at the recent 2014 EAPD Congress Meeting (Weerheijm 2015).

To address the shortcoming in MIH prevalence studies, Ghanim and colleagues created a MIH and/or HSPM scoring method or grading system (See Figure 8) (Ghanim et al. 2015). It is the only available epidemiological tool specific to MIH and/or HSPM for diagnosis and data collection. This measurement tool combined Weerheijm’s Judgement Criteria for MIH with Clarkson’s mDDE in order to differentiate MIH/HSPM from other enamel defects. There are two data collection forms, short and long (Ghanim et al. 2015). The short form is designed for MIH/HSPM screening: only the collection of first permanent molars, permanent incisors and secondary primary molars were recorded. The long form is designed for epidemiological research and involved the assessment of the entire dentition, as recommended by EAPD (See Figure 9) (Ghanim et al. 2015). Both forms require examiners to document defects at the tooth level in terms of eruption status and at the surface level in terms of clinical status (type of enamel defect) and lesion extension. The recording of all teeth in the long form gathers data to help prove or disprove if hypomineralisation is more common in first permanent molars, incisors and second primary molars than other teeth. In other words, data obtained from the long form will be the first step in determining if MIH/HSPM is a distinct clinical entity.
**Eruption status**

A = not visible or less than 1/3 of the occlusal surface or the crown length of incisor is invisible

### Clinical status criteria

<table>
<thead>
<tr>
<th>Description</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>No visible enamel defect</td>
<td>1</td>
</tr>
<tr>
<td>Diffuse opacities</td>
<td>11</td>
</tr>
<tr>
<td>Hypoplasia</td>
<td>12</td>
</tr>
<tr>
<td>Amelogenesis imperfect</td>
<td>13</td>
</tr>
<tr>
<td>Hypomineralisation defect (not MIH/HSPM)</td>
<td>14</td>
</tr>
<tr>
<td>Demarcated opacities</td>
<td>2</td>
</tr>
<tr>
<td>White/creamy demarcated opacities</td>
<td>21</td>
</tr>
<tr>
<td>Yellow/brown demarcated opacities</td>
<td>22</td>
</tr>
<tr>
<td>Post eruptive breakdown (PEB)</td>
<td>3</td>
</tr>
<tr>
<td>Atypical restoration</td>
<td>4</td>
</tr>
<tr>
<td>Atypical caries</td>
<td>5</td>
</tr>
<tr>
<td>Missing due to MIH/HSPM</td>
<td>6</td>
</tr>
<tr>
<td>Cannot be scored*</td>
<td>7</td>
</tr>
</tbody>
</table>

### Lesion extension (for 2-6)

<table>
<thead>
<tr>
<th>Description</th>
<th>I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 1/3 of tooth surface affected</td>
<td></td>
</tr>
<tr>
<td>At least 1/3 to 2/3 of tooth surface affected</td>
<td>II</td>
</tr>
<tr>
<td>At least 2/3 of tooth surface affected</td>
<td>III</td>
</tr>
</tbody>
</table>

**Instructions**

Score a tooth surface on MIH/HSPM if at least 1/3 or more of the tooth surface is available, otherwise, use Code A.

Score lesions that are greater than 1mm in diameter.

Circle to the tooth you are scoring on the charting sheet.

Record the clinical status and then lesion extension if applicable, “clinical status, lesion extension”.

Scores 2…7 for first permanent molars, incisors and second primary molars only.

Score non-MIH/HSPM lesion before MIH/HSPM if both lesions exist per surface.

Score the more severe rating if more than one MIH/HSPM lesion exist per surface.

Score the less severe rating if uncertain.

*Only for first permanent molars, incisors and second primary molars, cause of extensive coronal breakdown unknown.

---

Fig 8: Grading system for MIH and/or HSPM diagnosis has two components (scoring and data collection). The scoring/diagnosis incorporates the *Judgement Criteria* for MIH diagnosis, other developmental defects of enamel and lesion extension. All enamel lesions of at least 1mm in diameter on first permanent molars, incisors and second primary molars will be given a clinical
score (2-7) and lesion extension (I, II, III). Guidelines for completing the chart and diagnosis of lesions are provided. Source: (Ghanim et al. 2015)

![Fig 9](image)

**Fig 9.** The long data collection form requires examiners to record lesion type and extension for all teeth on all surfaces. Source: Adapted from Ghanim (Ghanim et al. 2015)

### 1.9 Treatment

Treatment for MIH affected teeth includes alleviation of discomfort, restorations, and extractions with or without orthodontic treatment. The decision as to which of these option(s) is indicated for particular patients depends on severity of hypomineralisation, presenting symptoms, patient’s dental age and the family’s expectations. Surveys conducted in Europe, Australia and New Zealand indicated that paediatric dentists and general dentists were not confident in MIH diagnosis and treatment despite seeing this condition in their practices (Weerheijm, Mejare 2003; Crombie et al. 2008). In 2009, the EAPD held a MIH consensus conference that was summarised in the 2010 EAPD policy document, entitled “Best Clinical Practice Guidance for clinicians dealing with Molar-Incisor-Hypomineralisation (MIH) (Lygidakis et al. 2010). Clinicians evaluated MIH severity (mild, severe) and patient’s dental development (early mixed, late mixed, full permanent) in selecting a treatment plan (See Figure 10). Mild cases were described as demarcated enamel opacities without enamel breakdown with possible sensitivity to external stimuli such as air/water but not to brushing. Severe cases were demarcated opacities with enamel breakdown, caries and associated with persistent or constant sensitivity.
<table>
<thead>
<tr>
<th>Severity</th>
<th>Early mixed</th>
<th>Late mixed</th>
<th>Full permanent</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mild</strong></td>
<td>Prevention</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adhesive + sealants for restorations</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Resin composite restoration</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Microabrasion, bleach + sealants for anterior</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Severe</strong></td>
<td>Prevention &amp; symptom control</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adhesive + sealants for posterior</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Microabrasion, bleach + sealants for anterior</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glass ionomer restorations</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Composite restorations</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Preformed metal crowns</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Orthodontic extraction</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cast restoration</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig 10: The management of MIH as per EAPD guideline is based on lesion severity and stage of dental development. *Source:* Adapted from Lygidakis (Lygidakis et al. 2010)
Mathu-Muju and Wright created a decision tree for MIH short-term and long-term treatment management based on their 3-tier severity scale (See Figure 11). Both guidelines share similar treatment approach in that the severity of lesions determined the invasiveness of the treatment (Mathu-Muju, Wright 2006; Lygidakis et al. 2010). Additionally, EAPD and Mathu-Muju and Wrights’ guidelines are intentionally broad. There are no definitive treatment recommendations due to the wide range of MIH clinical presentations and lack of available evidence for MIH treatment. The majority of the studies on MIH treatment are observational or retrospective, do not have a control group and do not account for confounding variables such as patient age and MIH severity (Lygidakis 2010; Elhennawy, Schwendicke 2016). Systematic reviews of MIH treatment published in 2010 and 2016 concluded that there was insufficient evidence to make strong recommendations for treatment of MIH.
Fig 11: Decision tree in managing MIH. *Source:* Adapted from Mathu Muju & Wright (Mathu-Muju, Wright 2006)
For mild cases, preventive care include desensitizing agents, fluoride varnish and fissure sealants. Lygidakis proposed that a fluoride application as part of routine maintenance was beneficial in the early post-eruptive stage because hypomineralised areas were particularly vulnerable to acid attacks. Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) (MI-Paste®, GC Company, USA) improved enamel structure in hypomineralised molars in vivo and in vitro after daily tray applications of 20 minute duration for 3 years. Whether this microscopic difference translated to clinical improvement was unknown but this was the only study to support the recommendations of CPP-ACP use in hypomineralised teeth that was previously based on expert opinion (Baroni, Marchionni 2011).

Limited evidence on the retention of pit and fissure sealants on hypomineralised molars compared to healthy molars was inconclusive. One study showed that more sealants placed on MIH affected molars required earlier (18%) and more frequent (23%) replacement than controls but sealant type and method of placement were unknown (Kotsanos et al. 2005). Applying bond between etch and sealant application (70%) resulted in greater retention than etch and seal technique (26%) in a split mouth design on hypomineralised molars after 4 years (Lygidakis et al. 2009). There were no statistically significant differences observed for caries progression and enamel breakdown between the two sealant types. The study did not have a control group but the reported success rate for etching and bonding before sealant placement on hypomineralised molars was comparable to sealants placed on healthy molars. In a prospective 18-month study, there was no statistically significant difference in retention between 16 sealants placed without bonding on hypomineralised molars with demarcated opacities (62%) and 25 healthy molars (72%) (Fragelli et al. 2017). Sealant retention in this study was comparable to Lygidakis et al, and Kotsano et al (Lygidakis et al. 2009; Kotsanos et al. 2005). Mathu-Muju and Wright recommended a 60 second pre-treatment with 5% sodium hypochlorite prior to etching and bonding (Mathu-Muju, Wright 2006). The intent of 5% sodium hypochlorite pre-treatment was to remove excess enamel protein found in hypomineralised teeth to enhance etching and bonding (Mathu-Muju, Wright 2006). Subsequent laboratory investigation did not corroborate this recommendation as there was no difference in the quality of sealant tags between etch and seal and bleach-etch-seal technique (Gandhi et al. 2012). Retention of bonded pit and fissure was comparable between MIH and healthy molars but there were no studies measuring caries development as the outcome. Additionally, no evidence exists to show the benefit of bonded pit and fissure sealants on molars affected by MIH.
Investigations on intra-coronal restorations for hypomineralised teeth focused on restoration material choice and more specifically the location of preparation margins for acid etch resin (AER) restorations.

For the restoration of MIH molars, two retrospective studies compared restorative materials including amalgam, acid etched resin (AER), compomer and glass ionomer cement (GIC) and two prospective studies assessed the longevity of AER restorations. GIC was not recommended for use in stress bearing areas on molars affected by MIH and could only be considered as an intermediate restoration (Lygidakis et al. 2010). Despite these recommendations, there were no studies demonstrating the benefit of GIC as a temporary restorative material and only retrospective studies for its use as a permanent restoration for hypomineralised molars.

Mejare et al. conducted a retrospective study (n=76 patients, 185 teeth) of 8-17 year-olds who received dental treatment for their hypomineralised molars with follow-up at mean age of 18 years-old (Mejare et al. 2005). Forty-two percent of molars were described to have enamel breakdown. Quality of restorations was scored based on a binary scale of good/acceptable (minor gaps or marginal staining) or unacceptable (major gaps and exposed dentin > 2 mm²). The median duration of restorations was 5.2 years. The proportion of good/acceptable restorations based on material choice (total number placed) was 49.2 GIC (63), 64.3% compomer (14), 78.1% amalgam (32) and 85.36% AER (34). Five teeth required subsequent root canal treatment and nine teeth received extra-coronal restorations.

Kotsano et al. reported lower success rates in their retrospective study for amalgam (38.9%) and AER (74.6%) compared to treatment on healthy teeth. Study participants with MIH were 11 times more likely to receive restorative intervention than their matched healthy controls for both composite and amalgam restorations. Retreatment of amalgam restoration was more than twice as likely as AER for MIH-affected teeth. This study and several other retrospective studies supported the use of AER versus amalgam in restoring hypomineralised molars due to superior bonding in atypical outline forms (Lygidakis 2010; William et al. 2006b). The findings should be interpreted with caution as Kotsano et al did not specify on the pre-existing conditions of the hypomineralised teeth and the indications and procedure for each treatment material.

Lygidakis and colleagues reported good/acceptable results for 2 or 3-surface AER restorations placed in 8-10 year-olds (Lygidakis et al. 2003). A total of 52 restorations were placed, including restorations for 21 teeth that had failing amalgam restorations. Evaluators adopted Cvar and Ryge clinical criteria in assessing the restorations at follow-up (Cvar, Ryge 2005). This was a
commonly used scale to assess the aesthetics and functionality of restorations in terms of colour-match/surface appearance, marginal discoulouration, anatomic form, marginal adaptation and secondary caries on a three tier scale (A = all standards for restoration met, B = restoration needed further observation but defect was acceptable, C = restoration was unacceptable) (Lygidakis et al. 2003). At 4-year follow-up, 49 restorations were available for assessment and they were all deemed to be clinically and radiographically acceptable. Colour match was the only factor that was rated as significantly less satisfactory (grade B) than baseline at each annual evaluation. There were no significant differences for surface appearance, marginal adaptation, anatomic form and secondary caries. The 100% clinically acceptable outcome for AER restorations reported in this study (Lygidakis et al. 2003) was higher than the previously reported success rate of 85.3% after 5 years and 74.4% after 4 years of follow-up (Mejare et al. 2005; Kotsanos et al. 2005). Possible reasons include case selection (2-3 surface restorations), treatment conditions and operator technique. Neither of the retrospective studies specified the initial condition of the tooth or treatment protocol, making it a challenge to compare outcomes. Two approaches for restoration outline form for hypomineralised teeth include complete removal of all defective enamel or removal of only porous or soft enamel. The first approach ensures bonding to healthy enamel at the expense of greater coronal structure loss. The second approach preserves more tooth structure but bonding to hypomineralised enamel can result in compromised bond strength (William et al. 2006b). Microshear bond strength was significantly higher when an all-etch single-bottle adhesive or self-etching primer adhesive was applied to healthy enamel than to hypomineralised enamel (William et al. 2006a). No significant difference was found between the two bonding systems. The dominant failure mode in hypomineralised enamel was cohesive failure of the enamel that was explained by the inherent weakness of hypomineralised enamel. This was further substantiated by the disorganised and porous enamel-adhesive interface seen under the SEM (William et al. 2006a).

De Souza et al. conducted an 18-month prospective randomized control trial comparing self-etching adhesive with total-etch adhesive bonding systems on 41 hypomineralised molars in 6-8 year-olds (de Souza et al. 2017). Restorations were assessed clinically at 6-month intervals in accordance with the World Health Organization (WHO) oral health survey including: anatomical form, marginal adaptation, surface texture, marginal discoulouration, retention and secondary caries. The success rates for the two systems were not statistically significantly different, 68% for self-etching and 54% for total-etching. Additionally, there were no statistically significant
differences in any of the WHO evaluation criteria. Samples in this study consisted of severe cases of MIH with PEB that may explain the lower success rate of AER compared to previously mentioned studies. Thus, evidence on the benefit of sealants on hypomineralised molars and type of sealant is inconclusive.

Currently, there are no studies comparing the longevity of intracoronal restorations among molars affected by caries versus molars affected by hypomineralisation. In a review by Hunter, he concluded a possible link between age of patient during restoration placement and longevity of restoration. AER occlusal restorations in individuals under the age of 8 years-old had a 59% survival rate compared 69-74% for individuals aged between 15-20 years-old (Hunter 1985). Similarly Bernardo found that 10% of restorations failed in 8-12 year-olds due to recurrent caries (67% amalgam, 88% AER) and the failure rate was higher with larger surface restorations (Bernardo et al. 2007). From the available research, it appears that the outcomes for restorations placed on molars affected by caries and mild MIH are comparable. However, severe cases of MIH will have worse prognosis than mild cases due to increased number of afflicted tooth surfaces.

According to the EAPD guidelines, the recommended treatment for hypomineralised molars with extensive PEB, categorized as severe MIH, is extracoronal restorations such as stainless steel crowns (SSC). In a 2-year prospective split mouth study, 18 out of 19 SSC placed in 6-13 year-old participants with severely hypomineralised molars demonstrated no difference in longevity and restoration quality when compared to cast metal crowns (Zagdwon et al. 2003). Criteria for longevity included retention of crown, absence of subsequent caries or pulp pathology and no extraction due to defective restoration. Criteria for quality included proximal contacts, occlusion, crown positioning, margins and surface coverage. A retrospective study of 41 gold and ceramic crowns on first permanent molars with developmental defects of enamel placed on 6-8 year-old participants showed retention, excellent marginal adaptation, maintenance of tooth vitality and absence of secondary decay at 2-5 year reassessment (Koch, Garcia-Godoy 2000).

Patients with severely hypomineralised molars often experience a cycle of restorative care followed by replacement of restorations (Mathu-Muju, Kennedy 2016). Mathu-Muju and Kennedy approximated the total cost of restoring first permanent molar to be 2307 USD from
SSC (292 USD), permanent crown (1050 USD), root canal treatment (965 USD) and additional 3000 USD if treatment failed and an implant was required (Mathu-Muju, Kennedy 2016). There may be additional costs associated with replacement of restorations, sedation and more importantly, the intangible costs associated with multiple dental treatments for the patient. These include time away from school/work and its potential effect on school performance, time away from work for the guardians and transportation costs. The reported US national average for orthodontic treatment by a general practitioner is $5130 and higher if treatment is performed by orthodontic specialists (Mathu-Muju, Kennedy 2016). In the management of severely compromised first permanent molars, extraction followed by orthodontic treatment may be a more cost-effective option than the dental work required to retain the teeth (Lygidakis et al. 2010; Mathu-Muju, Wright 2006; Mathu-Muju, Kennedy 2016; Ong, Bleakley 2010). The second and third permanent molars can assume the position and function of first and second permanent molars respectively during early mixed dentition stage.

All MIH treatment guidelines offer minimal information on the timing and consequences of first permanent molar extractions despite proposing it as a treatment option for severely compromised first permanent molars. First permanent molar extractions are avoided due to their importance in anchorage and their overall goal of preserving complete dental arch and maintaining occlusion. If extractions are inevitable, appropriate timing as defined by age of patient or bifurcation calcification of second permanent molars, could minimise the consequences of mesial tipping of the second permanent molar, incomplete space closure, alveolar ridge atrophy, and over-eruption of the opposing molar. From the early studies by Thilander et al. in 1970 to the recent study by Wu et al. in 2017, evidence for the ideal timing of first permanent molars is poor (Thilander, Skagius 1970; Wu et al. 2017). For patients with severe MIH, management of pain or infection may take precedence over considerations for space closure. Thus, treatment may mean extraction of the first permanent molar at an inopportune time. Mejare et al. found that at 18 years-old, 87% of individuals (n=32) who had 1-4 first permanent molar extracted were considered to have good or acceptable space closure that was not defined in the study (Mejare et al. 2005). The number of extracted first permanent molars was significantly correlated with number of severely affected molars and not the patient’s occlusion. At follow-up, there were no statistically significant differences in occlusion or midline deviation between patients with or without first permanent molar extractions (Mejare et al. 2005).
Preservation of the dental arch is not to be the sole factor in treatment planning for patients with MIH. More specialists than general dentists had concerns about the effect of MIH on patients’ quality of life including pain, appearance, anxiety, number of dental visits and days missed at school (Kalkani et al. 2016). Factors to consider prior to rendering treatment include practicality of treatment, prognosis or restorative care compared to extractions and anticipated short term and long term costs for the families when deciding to extract or restore a severely affected first permanent molar.

Few studies investigated the treatment of hypomineralised incisors. Treatment recommendations for white, yellow and brown opacities were all based on studies for fluorosis and post-orthodontic white spots that have different enamel characteristics than MIH. Proposed treatment options include microabrasion with abrasive paste and 18% hydrochloric acid, bleaching with 10-38% carbamide peroxide or 5% sodium hypochlorite, pumice and etching with 37.5% phosphoric acid, and etch-bleach-seal. Hypomineralised lesions were also treated by resin composite veneers with or without opaque adhesive (Lygidakis 2010).

Survey results from 606 Norwegian general dentists indicated that there were differences between what clinicians elected to do and what was recommended by the MIH guidelines (Kopperud et al. 2016). The participating clinicians had different opinions in treating a mild case, defined as asymptomatic intact hypomineralised molar and a severe case, defined as a sensitive hypomineralised molar with PEB (Kopperud et al. 2016). Fifty-four percent of clinicians chose to apply fluoride varnish on the mild case and 58% chose to place a GIC restoration in the severe case. According to the MIH guideline, pit and fissure sealants were recommended for mild hypomineralised molars while SSC or extractions were recommended in severe cases. Only 11% of dentists elected for SSC and 6% for extraction (Kopperud et al. 2016). The survey pool consisted of general dentists who may not be as familiar with use of SSC as paediatric dentists in the treatment of MIH or be aware of a MIH treatment guideline. The results of a 2016 UK survey about views and experiences of MIH by general and specialist dentists may explain why clinicians did not treat as per guidelines. Clinicians were not confident in their ability to diagnose MIH and found it difficult to distinguish it from other developmental defects of enamel (Kalkani et al. 2016). General dentists were often more unclear about MIH prognosis than specialists but both groups shared concerns about aesthetics, sensitivity, occlusion and patient behaviour.
Further MIH education is necessary to educate clinicians that treatment is not the same as for other developmental defects of enamel.

1.10 Caries

Children with MIH require more dental treatment and retreatment than children without MIH (Americano et al. 2017). There is weak evidence that children with MIH are more likely to have dental caries than children without MIH (Americano et al. 2017). The surface of hypomineralised enamel is more susceptible to bacterial ingress and the reported sensitivity experienced by patients can hamper regular oral hygiene. Between the period of 2005-2014, 17 cross-sectional and cohort studies from different geographic areas examined the association between MIH, as defined by EAPD criteria, and caries rate as defined by DMFT/dmfs (Americano et al. 2017). The studies ranged in sample size, age of participants (6-18 years-old) and the teeth that were examined (complete dentition or just the first permanent molars). Despite using standardized methods for diagnosing MIH and caries, differences in study methodologies hampered the comparability of results. The authors noted that while 3 out of the 17 studies demonstrated a 2.1-4.6 OR for increased caries risk. Overall, the quality of evidence for the included studies was low as most scored between 3-5 out of an 8 point modified Newcastle-Ottawa Quality Assessment Scale, assessment tool modified for cross-sectional and cohort studies (Americano et al. 2017). Similar to other aspects of MIH research the quality of the evidence was limited, in establishing an association between caries risk and MIH.

To our knowledge, there are no published studies on the prevalence of enamel hypomineralisation in North America. The goal of this study is to determine the prevalence of enamel hypomineralisation in dental patients at The Hospital for Sick Children in Toronto, Canada.
2 Objectives

1. To determine the prevalence of enamel hypomineralisation among new patients in the Department of Dentistry at The Hospital for Sick Children (SickKids) in Toronto.

2. To determine the presentation patterns of MIH and HSPM by number of first permanent molars affected, number of permanent incisors affected and number of primary second molars affected.

3. To determine the presentation patterns of MIH and HSPM by surface affected (buccal, palatal/lingual, incisal/occlusal), number of surfaces affected and size of lesion.
Chapter 3

3 Materials and Methods

3.1 Compliance with Ethical Standards

The Research and Ethics Board of SickKids approved the study protocol (REB #1000052479 Enamel Hypomineralisation: Prevalence and Presentation Patterns Among Patients in the Department of Dentistry at The Hospital for Sick Children).

3.2 Sample Size Calculation

The reported prevalence for MIH and HSPM were 2.8 to 44 percent and 0 to 9 percent, respectively. Assuming an enamel hypomineralisation prevalence of 5%, the minimum sample size needed was 429 participants for 96% confidence level with +/-2% precision.

3.3 Participants

The sample comprised of new patients who presented to the Department of Dentistry at SickKids during the study period. A new patient was defined as a patient who had never been seen in the Division of Paediatric Dentistry. Eligibility criteria to be seen at SickKids Dentistry include: patients with medical comorbidities birth - 17 years, healthy children under the age of 3 years of age with severe early childhood caries, for assessment of eligibility for the Ontario Ministry of Health and Long Term Care Cleft Lip & Palate/Craniofacial Dental Program and for walk-in emergency care.

3.3.1 Inclusion and Exclusion Criteria

To be eligible for the study, consent and/assent must be obtained. Participants who were deemed to have full understanding of the study and its implications provided direct consent. When participants were unable to provide full consent, their consent was obtained in the form of an assent. In these cases, the consent of guardians was also obtained. Participants had at least one erupting second primary molar and/or first permanent molar and a minimum of 1/3 of the clinical crown must be erupted for inclusion.
Participants who did not meet the inclusion criteria were not eligible for the study. Reasons included lack of consent and/or assent, inability to cooperate for a dental exam, and no clinically visible first permanent molar, incisor and/or second primary molar, due to patient’s age and not due to a history of extraction.

3.4 Training and Calibration

Examiners for the study consisted of eligible paediatric dental staff members and a graduate paediatric dental student (Y.W.). The first step was to educate the examiners on MIH/HSPM and the grading method used for this study for the diagnosis and recording of enamel hypomineralisation (Ghanim et al. 2015). This was done on an individual basis between Y.W. and each examiner (See Appendix 1 – PPT).

The second step was to calibrate the examiners by using 20 intra-oral clinical images¹ (See Appendix 2 – calibration exercise). The 20 selected images represented different clinical presentations of MIH and HSPM as well as other developmental defects of enamel. Y.W. held two calibration sessions, approximately 2 weeks apart, with each examiner. During each session, examiners assessed 20 images of enamel lesion displayed on an 11-inch MacBook Air® computer. Examiners scored each lesion in accordance to the grading system used in this study. Due to the inherent differences between a digital image and a clinical exam, verbal clarifications were made about presence of enamel structure loss or if a lesion was sticky, suggestive of caries. All responses were collected on a calibration sheet and were subsequently categorized into MIH and non-MIH lesions (See Appendix 3 – Calibration sheet). Two separate inter-rater and intra-rater kappa values were calculated for recognition of lesion type (MIH vs. non-MIH) and lesion extension. For discerning lesion type, inter-rater agreement was 0.65-0.73 and intra-rater was 0.76, which were considered substantial on the Landis and Koch-Kappa’s Benchmark Scale (Landis, Koch 1977). Agreement was moderate in assessing lesion extension with inter-rater agreement being 0.4-0.5 and intra-rater agreement being 0.56-0.76.

¹ Use of images was licensed from the University of Melbourne
3.5 Examination

Examiners completed a quality assurance form that was a checklist for eligibility, consent, and other background information including date of visit, sex and primary medical diagnosis (See Appendix 4 – quality assurance form). All examinations were performed in a dental operatory with mirror and explorer. Examiners performed prophylaxis on teeth with a slow speed hand-piece or toothbrush if deemed necessary for accurate diagnosis. All tooth surfaces were kept moist during examination. Dental assistants recorded the grades given by the examiners on a data collection form that included the long form created by Ghanim et al. with accompanying instructions (Ghanim et al. 2015) (See Appendix 5 – data collection form).

3.6 Statistical Analysis

Study data were collected and stored using REDCap™ hosted at SickKids (Vanderbilt University, Nashville, Tennessee, USA) and analysed by Statistical Analysis Software, SAS© for Windows (SAS Institute, Cary, North Carolina, USA). Descriptive statistics were calculated for qualitative variables, represented by proportions. Quantitative variables were represented by means with confidence intervals. Chi-square tests determined significant differences between qualitative variables. Odds ratios between MIH and HSPM were used to determine if HSPM was prognostic for MIH. Predictive values, likelihood ratio and odds ratio were calculated. The level of significance was set at p<0.05.
Chapter 4

4  Results

4.1  Participants

A total of 1024 patients were scheduled as new patients during the study period of May 30, 2016 and Feb. 22, 2017. Of those, 856 patients were screened for eligibility and 429 patients met the inclusion criteria (See Figure 12). Reasons for exclusion were clinically missing all second primary molar or first permanent molar (n=112), not a new patient (n=165), inability to perform a dental exam by examiner (n=87) and declined participation (n=36).

Fig 12: Participant screening and enrolment as per inclusion and exclusion criteria

*screened number of participants include all participants that were scheduled as “new patients” on daily clinic schedule

The final sample size consisted of 120 healthy participants and 309 participants with at least one medical co-morbidity. The sex distribution of participants was 248 males (58%) and 181 females.
(42%). Three hundred and sixty-five participants (85%) had at least one second primary molar and 233 (54%) had at least one first permanent molar.

4.2 Quality Assurance

The author ensured that consent, quality assurance and data collection forms for each participant were complete. If uncertainty could not be resolved with the original examiner or incomplete records, the participant was excluded from the study. All data was entered into REDCap™ database that was subsequently verified by another study team member.

4.3 Prevalence

Among the 429 participants, there were 29 participants with MIH and 19 with HSPM (See Table 1). Three individuals were diagnosed with MIH and HSPM. Among the participants diagnosed with MIH and/or HSPM, 29 (60%) were males and 19 (40%) were females. The prevalence of MIH was 12.4% (95% CI, 8.5-17.5%) among participants with at least one first permanent molar. Thirteen of the 29 participants with MIH had hypomineralisation defects on first permanent molars and incisors. A total of 42 incisors, 116 first permanent molars and 36 second primary molars had hypomineralisation. The prevalence for HSPM was 5.2% (95% CI, 3.1-8.1%) among participants with at least 1 second primary molar. The prevalence of HSPM and MIH was 1.9% (95% CI, 0-4.1).

Table 1 Prevalence based on selected sample groups

<table>
<thead>
<tr>
<th></th>
<th>Number (%Prevalence, 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIH</td>
<td>29 (12.4, 8.5-17.4)</td>
</tr>
<tr>
<td>MIH with incisor involvement</td>
<td>13 (5.6, 2.6-8.5)</td>
</tr>
<tr>
<td>HSPM</td>
<td>19 (5.2, 3.1-8.1)</td>
</tr>
<tr>
<td>MIH and HSPM</td>
<td>3 (1.9, 0-4.1)</td>
</tr>
<tr>
<td>Other developmental defects of enamel (n=429)</td>
<td>41 (9.6, 6.9-12.7)</td>
</tr>
<tr>
<td>MIH/HSPM and other enamel defects</td>
<td>0</td>
</tr>
</tbody>
</table>
4.4 Distribution of MIH and HSPM

4.4.1 Number of teeth involved

The frequency of first permanent molars and incisors affected by MIH and second primary molars affected by HSPM are shown in Figure 13. Additionally, first permanent molars were most frequently affected, followed by maxillary permanent incisors (See Fig. 13). The upper left second primary molar was least likely affected by HSPM. The mean number of first permanent molars affected in participants with MIH was 2.5. The mean number of second primary molars affected in participants with HSPM was 1.5. There was no significant difference between individuals with, 1, 2, 3 or all 4 first permanent affected by MIH (p=0.51). Similarly, there was no significant difference noted in the number of second primary molars involved in those with HSPM (p=0.25). Individuals with HSPM were significantly more likely to have 2 molars involved than individuals with MIH (p=0.0).

Fig. 13: Distribution of hypomineralised first permanent molars, incisors and second primary molars affected by MIH and HSPM

4.4.2 Number of surfaces involved and lesion location

Hypomineralisation was recorded on up to three surfaces (buccal, occlusal/incisal, palatal) for each tooth. The majority of first permanent molars, incisors and second primary molars were affected by MIH and HSPM had lesions affecting only one surface (See Figure 14). Single
surface involvement was significantly more common than defects affecting 2 or 3 surfaces in MIH and HSPM (p<0.0001). Additionally, HSPM was significantly more likely to affect one surface compared to teeth affected by MIH (p=0.03). The most common location for hypomineralisation in MIH and HSPM was the buccal surface but this was not statistically significant compared to palatal or occlusal/incisal (See Figure 15).

Fig. 14: Number of surfaces involved in teeth affected by MIH and HSPM. Lesions affecting one surface are more common than lesions spanning two or three surfaces (p<0.0001). There are more cases of HSPM than MIH single surface lesions (p=0.03).

Fig. 15: Location of MIH and HSPM lesions
4.4.3 Clinical presentations

White demarcated opacities were the most common presentations of MIH and HSPM (Fig. 16). Three participants had PEB, another three had atypical caries and two had atypical restorations. Among those with MIH, 12 participants (41%) had more than one clinical presentation observed on the surfaces of their first permanent molars and incisors. No participant with HSPM exhibited a combination of defects. There were no cases of extractions due to MIH and HSPM. There were no statistically significant differences between the number of HSPM and MIH participants with white (p=0.84) and brown opacities (p=0.316). Significantly more surfaces affected by MIH were brown (p=0.04) and fewer surfaces were PEB (p=0.002), compared to surfaces affected by HSPM. In individuals with HSPM, no surfaces were noted to have atypical restorations or atypical caries.

![Clinical presentations chart](chart.png)

Fig. 16: Clinical presentations of MIH and HSPM. Brown opacities are significantly more common in MIH than HSPM (p=0.04). PEB is significantly more common in HSPM than MIH (p=0.002).

4.4.4 Lesion size

In addition to location and number of surfaces involved, the sizes of lesions were also documented. Hypomineralised defects were categorized based on: I (<1/3 of clinical crown

52
surface), II (1/3-2/3 of clinical crown surface) and III (1/3< of clinical crown surface) (See Figure 17). Defects observed to be <1/3 of clinical crown surface were significantly more common on first permanent molars, incisors and second primary molars (p<0.0001). Size I lesions on HSPM were significantly more common that defects found on MIH (p=0.0002).

Fig. 17: Size of MIH and HSPM lesions Size I (<1/3 of clinical crown surface) lesions were significantly more common than sizes II (1/3 to 2/3 of clinical crown surface) and III (>2/3 of clinical crown surface) (p=0.0001). HSPM size I lesions were significantly more common than MIH size I lesions (p=0.0002)

Figures 18 and 19 show the distribution of lesion size based on clinical presentations of MIH and HSPM. All three sizes of hypomineralisation defects were observed on the clinical crown of
MIH teeth when they presented as white, brown opacities and PEB. All MIH surfaces with atypical restorations were larger than 2/3 of clinical crown. For second primary molars with PEB, the majority of lesions were between 1/3 to 2/3 of clinical crown surfaces.

**Table 2** Distribution of MIH lesions by size and clinical presentation.

<table>
<thead>
<tr>
<th>Size</th>
<th>White (%)</th>
<th>Brown (%)</th>
<th>PEB (%)</th>
<th>Ares (%)</th>
<th>Acar (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>76 (72)</td>
<td>19 (48)</td>
<td>2 (14)</td>
<td>0</td>
<td>5 (50)</td>
</tr>
<tr>
<td>II</td>
<td>17 (16)</td>
<td>8 (20)</td>
<td>3 (21)</td>
<td>6 (100)</td>
<td>5 (50)</td>
</tr>
<tr>
<td>III</td>
<td>12 (12)</td>
<td>13 (32)</td>
<td>9 (65)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Ares = atypical restoration, Acar = atypical caries

**Table 3** Distribution of HSPM lesions by size and clinical presentation

<table>
<thead>
<tr>
<th>Size</th>
<th>White (%)</th>
<th>Brown (%)</th>
<th>PEB (%)</th>
<th>Ares (%)</th>
<th>Acar (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>27 (90)</td>
<td>3 (75)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>1 (3)</td>
<td>1 (25)</td>
<td>8 (73)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>III</td>
<td>2 (7)</td>
<td>0</td>
<td>3 (7)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Ares = atypical restorations, Acar = atypical caries

4.5 MIH and HSPM

HSPM was not a significant prognostic factor for MIH (OR=2.8, 95% CI, 0.76-10.12) (p=0.11).
Chapter 5

5 Discussion

There have been a large number of MIH and HSPM epidemiological publications since the introduction of the EAPD Scale. Early studies originated mostly in Europe and Australia but in the past 5 years interest in MIH has risen in many areas of the world including Africa and Asia (Elfrink et al. 2015). Representation from North America was visibly absent. To the knowledge of the author, this study is the first epidemiological study on enamel hypomineralisation from North America. Our study recorded a prevalence rate of 12.4% for MIH and 5.6% for MIH with incisor involvement and 5.2% for HSPM. These values fall within the range reported from existing publications. This further answers the question that MIH is a worldwide and not a region specific phenomenon (Weerheijm 2015).

The global MIH prevalence was 14.2% from a meta-analysis of 70 prevalence studies but authors noted substantial heterogeneity in data. Inconsistencies in MIH/HSPM epidemiological research in terms of demographics, examination settings and protocols compromised the comparability of study outcomes. The EAPD promoted the need of a “worldwide standardised scoring and calibration system” to allow comparisons of MIH and HSPM prevalence values (Weerheijm 2008). Additionally, empirical evidence on the clinical presentation patterns of MIH and HSPM could determine if these two conditions are unique, related or no different from any other hypomineralisation defects found anywhere else in the dentition. The clinical implications include determining if MIH is a common clinical condition that more dentists should be concerned about and describing a standardised scoring and calibration system for diagnosis and treatment. Our study offered insight into the first two parts by determining the prevalence and distribution patterns of MIH and HSPM.

Our study was among the few that recorded details about the distribution of enamel hypomineralisation at the surface level and the only one to date, to use a grading and data collection form endorsed by the EAPD (Ghanim et al. 2015; Petrou et al. 2015; Mittal et al. 2014). Thus, direct comparisons with the present study’s outcomes of MIH and HSPM distribution patterns in terms of number of teeth involved and clinical presentations were not possible. In some studies, the occlusal surface of first permanent molars and buccal surface of
incisors were commonly affected by hypomineralisation (Mittal et al. 2014; Lygidakis et al. 2008; Petrou et al. 2015; Ghanim et al. 2011). In our study, the buccal surface was most common for first permanent molars, incisors and similarly in second primary molars for HSPM. Coloured or opaque pit and fissure sealants (PFS) on first permanent molars could mask possible hypomineralised defects. In our study lesions on the buccal surface were not along the developmental grooves that could have been masked by PFS but located on buccal bulges close to cusps. The average number of surfaces affected in MIH (1.5) was lower than previously reported studies (4.5 and 4.99) (Petrou et al. 2015; Mittal et al. 2014). Petrou et al. examined all five surfaces of a tooth unlike the present study’s protocol that asked for three surfaces, excluding mesial and distal. Only the lingual/palatal, occlusal/incisal and buccal surfaces of clinically present teeth were examined because they were easily visualized and the assumption was that children would have intact arches, hindering the ability to see mesial and distal surfaces.

Previously, a statistically significant positive correlation between number of surfaces involved and MIH severity was reported (Petrou et al. 2015). In this study, the authors defined severe lesions when there was a positive history of tooth sensitivity. In addition to patient symptoms, many investigators described mild cases as teeth with intact enamel or demarcated opacity and severe cases as teeth with compromised enamel including PEB, atypical restorations, and atypical caries. Additionally, teeth that were extracted due to hypomineralisation were also categorised as severe MIH. Applying this to our study, only 18% and 24% of recorded surfaces were severe for MIH and HSPM, respectively. The significantly greater number of coronal surfaces with PEB seen in primary secondary molars compared to permanent molars (p=0.002) could be explained by the smaller surface area of primary teeth. In an editorial response, Elfrink and colleagues agreed that hypomineralised defects on second primary molars were more likely to progress to PEB, atypical restorations, atypical caries, and possible extractions in older children, even at the recommended screening age of 5 years-old (Elfrink et al. 2016a). The use of SSCs for caries or large surface defects could explain the absence of atypical caries or restorations on second primary teeth. The present study protocol did not consider the use of SSC as atypical restoration as recommended in earlier studies because the common use of SSC in primary dentition could overestimate or underestimate the HSPM rate.

Documentation of lesion size is unique to this scale. Most hypomineralisation defects observed in this study were small, less than 1/3 of the tooth surface. The frequency of larger lesions as
defined by >2/3 of surface increased from white opacity (11%) to brown opacity (33%) to PEB (64%). This trend reflects data collected at the surface level and not at the individual level. A positive correlation between lesion size and severity could not be analysed because 14 PEB surfaces were seen in only 3 participants and of those, 12 surfaces originated from a single participant. Findings in our study further confirmed that severe cases of MIH/HSPM were rare whether this was defined by clinical presentation or lesion size.

The grading scale used in this study was an amalgamation of the FDI mDDE scale and the EAPD Judgement Criteria (Ghanim et al. 2015). There were two versions of the grading scale. The short version required the examination of only the teeth associated with MIH and HSPM: first permanent molars, incisors and primary second molars. The long version required the examination of all clinically present teeth and recording of all developmental defects of enamel, not limited to MIH and HSPM. This served two purposes. First, additional information on the size and location of hypomineralised lesions helped to discern if MIH and HSPM were localised conditions to specific teeth or on a spectrum of a more generalised condition of enamel hypomineralisation. Second, the systematic approach in examining the complete dentition reminded clinicians to consider all differentials for developmental defects of enamel and not to default to MIH or HSPM as the diagnosis. From our experience, there was a steep learning curve associated with completing the data collection forms. Examiners reported the scale was overly complex for diagnosing MIH and HSPM. The grading system was not intuitive and thus, some examiners expressed confusion and frustration. Ideally, an epidemiological tool should be simple and quick. Anything to the contrary potentially discourages use or worse, would compromise the integrity of the data when examiners complete it haphazardly.

Results of our study should be interpreted with awareness of its limitations. The total sample size of the study, n=429, comprised of all participants who had at least one primary second molar or first permanent molar. Subsets of participants with at least one first permanent molar and second primary molar were used to determine the study’s prevalence of MIH and HSPM, respectively. The accuracy of the prevalence value is limited to the point in time of the examination and may be an estimate of the true value because many participants did not have all four of their first permanent molars or second primary molars present. For this reason, the EAPD had recommended the age of MIH assessment to be 8-years-old. Implementing this specific age
inclusion criteria was unrealistic for collecting an adequate sample size within the study’s timeline.

Prevalence of enamel hypomineralisation was expected to be higher than the average population because 72% of our participants had at least one medical comorbidity. One study found significantly higher rates of MIH in children with HIV than those without, but the distribution of MIH presentation was the same (Andrade et al. 2017). While the etiology of MIH/HSPM remains unclear, perinatal events are hypothesized to disturb the amelogenesis of second primary molars, first permanent molars and incisors. Another interpretation of the data is that the prediction of enamel hypomineralisation based on perinatal medical events is an over simplification. The involvement of other systemic such as fever and amoxicillin usage and local factors such as genetic mutations of ion channels could explain what appears to be an inconsistent distribution of MIH/HSPM presentation patterns.

More studies that use the same methodology are needed to substantiate the findings of this study and to further show that MIH and HSPM are distinct clinical entities. Study designs with larger sample size and follow-up could show a potential relationship between HSPM and MIH. Only a select number of patients presented with what was previously considered as severe cases of MIH. Thus, very few individuals with severely affected first permanent molars pose a clinical concern. In addition to epidemiological research, the challenge continues to be in determining the right course of treatment as the presentation patterns of MIH and HSPM vary greatly in terms of number of teeth, size, location and clinical presentation of hypomineralised defects.
6 Conclusion

In conclusion, MIH prevalence was 12.4% (n=29) for FPM and 5.6% (n=13) for FPM and permanent incisors and 5.2% (n=19) for HSPM. This studied showed that MIH and HSPM were observed in a North American population and that the study’s sample of participants from Department of Dentistry at SickKids shared similar prevalence values of MIH and HSPM as found in other international epidemiological studies.

The average number of molars affected by enamel hypomineralisation was 2.5 for MIH and 1.5 for HSPM. There were no significant differences between the number of molars affected among MIH and HSPM participants. However, participants with HSPM had significantly more cases of having two affected molars than MIH.

The distribution pattern of MIH and HSPM at the surface level showed that on teeth affected by enamel hypomineralisation, single surface involvement was significantly more common than any of the three surfaces that were recorded. Single surface involvement was significantly more common in HSPM than MIH. There was no significant difference in the location of hypomineralised lesions when comparing the three recorded surfaces. Hypomineralised lesions were most frequently gauged to cover less than 1/3 of a tooth surface. This was significantly more common compared to the other two lesion size categories, 1/3 to 2/3 and greater than 2/3 of tooth surface. Second primary molars had significantly more defects of this size than first permanent molars and/or incisors.

 Majority of participants with MIH and HSPM presented with white opacities as described by MIH Judgement Criteria. For the other clinical presentations of MIH and HSPM, brown opacities were significantly more common on surfaces of MIH affected teeth and PEB was significantly more common on surfaces of MIH. Atypical restoration and atypical caries were only observed in MIH and HSPM. No participants in our study presented with a history of extraction of their first permanent molar, incisor or secondary primary molar due to enamel hypomineralisation.
References


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Oliver K, Messer LB Fau - Manton DJ, Manton Dj Fau - Kan K, Kan K Fau - Ng F, Ng F Fau - Olsen C, Olsen C Fau - Sheahan J et al. Distribution and severity of molar hypomineralisation: trial of a new severity index. (1365-263X (Electronic)).


Appendices

Appendix 1: Training Module

Enamel Hypomineralisation

Staff Training Module

Yili Wang, Masters of Science Project

Study Aims

1. To determine the prevalence of MIH/HSPM among new patients in the Department of Dentistry at The Hospital for Sick Children

2. To determine the presentation patterns of MIH/HSPM in terms of the number and surfaces of teeth affected
Molar Incisor Hypomineralisation (MIH)

• *At least* one *first permanent molar* affected with or without *permanent incisor* involvement

• Hypomineralised second primary molar (*HSPM*)

• Etiology unknown

(Cobourne et al, 2014)

Study Package

• Available in all new patient charts

• *Contents*
  - Eligibility check list
  - Consent forms
  - Definition/reference sheet
  - MIH/HSPM scoring form
Participant Eligibility

**Inclusion**
- New patients
- At least one erupted second primary molar, first permanent molar, and permanent incisor
- Cooperative for exam in clinic
- or will require treatment under GA
- Consent and/or assent obtained

**Exclusion**
- No teeth associated with MIH/HSPM
- Poor cooperation and does not require GA
- No consent and/or assent

---

**MIH/HSPM Scoring Form**

*Per tooth:*
1. Eruption status (A or circle tooth)

*Per surface:*
2. Clinical status(es) (0 – 7)
3. MIH/HSPM Lesion extension (I, II, III)
1. Eruption Status

• $A = \text{not visible or } < \frac{1}{3} \text{ occlusal surface or crown length visible}$

2. Clinical Status

• $0 = \text{no visible enamel defect}$
• $1 = \text{non-MIH/HSPM enamel defect}$
• $2 = \text{demarcated opacities}$
• $3 = \text{post-eruptive enamel breakdown (PEB)}$
• $4 = \text{atypical restoration}$
• $5 = \text{atypical caries}$
• $6 = \text{missing due to MIH/HSPM}$
• $7 = \text{cannot be scored}$
Non-MIH/HSPM Enamel Defect

11 = diffuse opacities
12 = hypoplasia
13 = amelogenesis imperfecta
14 = hypomineralised defects (not MIH/HPSM)

MIH/HSPM Enamel Defect

• 2. Demarcated Opacities
  • 21 = white or creamy
  • 22 = yellow or brown
MIH/HSPM Enamel Defect

3. Post-eruptive enamel breakdown (PEB)  4. Atypical restoration

5. Atypical caries  6. Missing due to MIH/HSPM

3. MIH/HSPM Lesion Extension

- For codes 2 – 6 only:

  - I = <1/3 tooth affected
  - II = 1/3 to 2/3
  - III = 2/3 <
MIH/HSPM Scoring Form

1. Circle around the tooth number
2. Eruption status (< or > 1/3) or cannot be assessed (7)
3. >1mm
4. Record clinical status and then, lesion extension (, )

Clarifications

- If there are more than one type of lesion, score nonMIH/HSPM first (1) and then MIH/HSPM lesions second (2-7)
- If uncertain, use less severe rating
- If there is more than one MIH/HSPM lesion on a surface, score the more severe presentation and visually combine all areas affected by lesion
Example 1

• Tooth 36
• Eruption status?
  – Yes, fully erupted
• Buccal surface
• Clinical lesion?
  – Demarcated white lesion and post eruptive breakdown
  – PEB (3)* more severe
• Lesion extension?
  – II (1/3 – 2/3)
• 3, II

Example 2

• Tooth 36
• Eruption status?
  – Yes, fully erupted
• Occlusal surface
• Clinical lesion?
  – Atypical restoration (4)
• Lesion extension?
  – III (>2/3)
• 4, III
Example 2

- Tooth 36
- Eruption status?
  - Yes, fully erupted
- Occlusal surface
- Clinical lesion?
  - Atypical restoration (4)
- Lesion extension?
  - III (>2/3)
- 4, III
Calibration Session 1

Yili Wang, Masters of Science Project
Photos are the property of Dr. Aghareed Ghanim
Melbourne Dental School
University of Melbourne

Case 1: 63V
Case 4: 11V

Case 5: 11V
Case 6: 41V

Case 7: 26O
Case 8: 16L

Case 9: 36B
Case 10: 16O

Case 11: 46
Case 12: 11V

Case 13: 16O
Case 14: 65P

Case 15: 11V
Case 16: 11V

Case 17: 12V
Case 18: 26P

Case 19: 46B
Case 20: 11V
Appendix 3: Calibration Exercise (Adapted from Ghanim, 2015)

Examiner’s initials:
Date:
Session: 1, 2 (circle one)

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### Appendix 1: ELIGIBILITY CHECKLIST

**Full Study Title:** Enamel hypomineralisation: Prevalence and presentation patterns among patients in the Department of Dentistry at The Hospital for Sick Children  

**Qualified/Principal Investigator (QI/PI):** Dr. Michael Casas  

**REB File #:**

**Participant Study ID:**

---

**Inclusion Criteria must match study protocol**

**INCLUSION CRITERIA (Answer all questions)**  
If the answer to any inclusion criteria is **NO**, do not enroll the participant into the study.

1. New patient to Dentistry  
   - YES ☐  
   - NO ☐
2. At least one erupted second primary molars, first permanent molars, and permanent incisors  
   - YES ☐  
   - NO ☐
3. Cooperative for exam in clinic or scheduled for a GA  
   - YES ☐  
   - NO ☐

---

**Exclusion Criteria must match study protocol**

**EXCLUSION CRITERIA (Answer all questions)**  
If the answer to any exclusion criteria is **YES**, do not enroll the participant into the study.

1. No teeth associated with MIH/HSPM  
   - YES ☐  
   - NO ☐
2. Poor cooperation and does not require GA  
   - YES ☐  
   - NO ☐

---

**Signature of Person who Assessed Eligibility**  
**Date (yyyy/mm/dd)**

**Signature of Qualified/Principal Investigator or delegate confirming eligibility**  
**Date (yyyy/mm/dd)**  
**Time:** ________________

*as delegated on signature and delegation log
Appendix 5: MIH/HSPM Data Collection Form (Adapted from Ghanim et al, 2015)

Study ID:

Dentist:

Date of initial examination:

Final date of examination:

Sex:

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Scoring Key

**Eruption Status**
A = Not visible or less than 1/3 of the occlusal surface or the crown length of incisor is visible

**Clinical Status**
0 = no visible enamel defect
11 = diffuse opacity
12 = hypoplasia
13 = AI
14 = hypomineralized defect (NOT MIH/HSPM)
21 = white/creamy demarcated opacity
22 = yellow/brown demarcated opacity
3 = quantitative defect (PEB/developmental)
4 = atypical restoration
5 = atypical caries
6 = missing due to MIH/HSPM
7 = Cannot be scored*

**Lesion Extension (only for MIH/HSPM)**
I = less than 1/3 affected
II = 1/3 – 2/3 affected
III = greater than 2/3 affected

**Instructions:**
1. Circle the teeth that are present and to be scored
2. Determine the eruption status
3. *If the tooth is too broken down, score 7
4. Examine each surface of each tooth (buccal, occlusal, palatal)
5. Score defect that are >1mm
6. Determine if the enamel defect is:
   - not MIH/HSPM (11-14)
   - MIH/HSPM related (2-6)
   *Refer to EAPD Evaluation Criteria for definitions*
7. If only the incisal surface of a MIH/HSPM tooth is affected, ignore the other surfaces.
8. Determine the extent of the defect per surface and if there are multiple defects, use the summation of their sizes