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ORIGINAL RESEARCH

Journal of Clinical Pediatric Dentistry

Investigation on the effects of long-term antibiotic therapy in sickle cell disease associated with molar-incisor hypomineralisation—a pilot study

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Abstract

The term Molar-Incisor Hypomineralisation (MIH) is used to describe hypomineralised defects of systemic origin that affect at least one of the first permanent molars and often involves the permanent incisors. Antibiotic therapy during amelogenesis may be associated with enamel hypomineralisation. By examining children with Sickle Cell Disease (SCD), who take prophylactic antibiotics daily from birth until age five, it may be possible to determine if there is an increased prevalence of MIH in this population. The aim of this study was to determine the effect of long-term antibiotic use on the prevalence and severity of MIH in children with SCD. In a prospective cohort pilot study over a period of seven months, children aged 7–17 years, with SCD at Boston Children's Hospital (n = 18) were examined for MIH. Information regarding peri-natal concerns, incidence of illness and antibiotic use were also collected. The results were compared to a group of control patients (n = 63) for prevalence and severity of MIH using Fisher's exact test. The patients with SCD, 4/18 (22%) taking daily antibiotics did not show a statistically significant greater prevalence of MIH compared to the control group, 24/63 (38%). There was no correlation between MIH and pneumonia, asthma, fever, flu, otitis media, breastfeeding, gender and birth weight. However, an association was noted between premature birth and MIH ($p \leq 0.05$). No correlation was found between long-term antibiotic use and higher prevalence of MIH in the SCD group compared to the control group. However, MIH may be more severe in those with a history of long-term antibiotics.

Keywords

Molar-incisor hypomineralisation; Sickle cell disease; Antibiotic use; Enamel defects; Prevalence

1. Introduction

Molar-Incisor Hypomineralisation (MIH) is defined as a qualitative defect of enamel of systemic origin of one to four first permanent molars, frequently associated with affected incisors [1]. The diagnostic criteria require at least one or more first permanent molars (FPM) to have one of the following characteristics: demarcated enamel opacity, post-eruptive enamel breakdown, an atypical restoration or an extraction due to MIH [1–3].

Clinically, the porous hypomineralised areas appear as white, yellow or brownish, well-defined opaque spots. These spots are weak, with a significantly lower tooth mineral density compared to unaffected enamel [4]. The affected areas can easily breakdown, especially on the occlusal surfaces of the teeth, which can then lead to caries [2]. Children with MIH are 2.1 to 4.6 times more likely to have caries in the permanent dentition than children without MIH [5].

The prevalence of MIH varies globally depending on the study population. Evidence from 70 studies has demonstrated a global prevalence of 14.2% with significant heterogeneity between studies [6]. South America was found to have the highest prevalence of MIH at 18%. In the United States of America (USA), there is a scarcity of research on prevalence of MIH. In a pilot study, Davenport and colleagues found the prevalence of MIH in a convenience sample in Milwaukee, to be 9.6% [7]. Similarly, a 9.6% prevalence was found in a group of patients from University of Pittsburgh [8]. In comparison, a conference paper reported a prevalence of 29.5% in a non-representative sample in South Texas [9]. Considering the population variations in the USA, there is clearly a need for further epidemiological research of MIH throughout the country.

The aetiology of MIH is not well understood. There is evidence of both genetic and epigenetic factors contributing to MIH [10–12]. The environmental and illness-based factors previously explored in the literature include childhood illness, prematurity, hypoxia at birth [13] and medication use, particularly the use of antibiotics [14]. Some studies have

demonstrated an association between antibiotic use as a child, in particular penicillin use and MIH [15, 16]. Children with a history of antibiotic intake in the first year of life are twice as likely to have MIH [16]. Recent *in-vivo* studies found that the administration of amoxicillin in juvenile mice induced enamel hypomineralisation by reducing the calcium/phosphate ratio in enamel and creating more intercellular spaces among maturational ameloblasts [17, 18].

However, the main drawback in previous studies, is the inability to determine if the cause of the enamel defect is the antibiotic itself or the illness that necessitated the use of the antibiotic [19–22]. There are no studies to date, that the authors are aware of, that have explored the prevalence of MIH in patients with a history of long-term antibiotic therapy in early childhood.

In order to differentiate between antibiotic use and acute childhood illness, this study examined patients with Sickle Cell Disease (SCD) taking prophylactic antibiotics. Sickle Cell Disease, is a multisystem disease, associated with episodes of acute illness and progressive organ damage, and is one of the most common severe monogenic disorders worldwide [23]. Patients with SCD have a defective haemoglobin or sickle haemoglobin (HbS) mutation resulting in an alteration in the shape of the red blood cells or "sickling". Sickled red blood cells become trapped predominantly in the slow flowing venular side of the microcirculation [23, 24]. Symptoms of SCD arise from chronic endothelial damage at the microvasculature level, especially in the spleen, thus predisposing patients to complications such as infection [23, 24]. Therefore, all SCD patients at Boston Children's Hospital are prescribed prophylactic penicillin, from birth through age five years. This makes the SCD patient group ideal for investigation of the effects of long-term antibiotic therapy on prevalence and severity of MIH.

The aim of our study was to determine the effect of long-term antibiotic use on the prevalence and severity of MIH in children with SCD compared to healthy controls and to evaluate previously suggested environmental and illness-based risk factors that may contribute to the prevalence of MIH in the patient population studied.

2. Materials & method

This prospective cohort pilot study consisted of children aged 7–17 years with SCD who have taken Phenoxymethylpenicillin (Penicillin VK) or antibiotic prophylaxis under the Boston Children's Hospital protocol. This protocol entailed the following: from birth to the third birthday: penicillin VK 125 mg twice a day, and from third birthday to fifth birthday: penicillin VK 250 mg twice a day. The control group consisted of healthy children 7–17 years of age attending the Boston Children's Hospital Dental Clinic. Patients who presented for their routine recall examinations or treatment at Boston Children's Hospital in the Paediatric Dental Department or Sickle Cell Disease Program were assessed based on the inclusion and exclusion criteria listed below.

The inclusion criteria for the SCD group included patients with SCD on antibiotics for at least the first three years of life; ages of $\geq$7 and $\leq$17 years; and the presence of at least one erupted first permanent molar. The inclusion criteria for the control group were the absence of a history of long-term antibiotic use (>2 weeks); the absence of a history of systemic disease/medical issues (*e.g.*, Cerebral Palsy, Down Syndrome) and the presence of at least one erupted first permanent molar.

The exclusion criteria for both groups included patients who were carriers of sickle cell trait; had a diagnosis of amelogenesis imperfecta, dentinogenesis imperfecta, fluorosis or other developmental defects of enamel and dentin; very low birth weight (1500 g) as defined by the World Health Organization [25] or had first permanent molars <1/3 erupted.

Once informed consent was obtained from the legal guardian and informed assent obtained from each research subject, the teeth were examined using the guidelines specified in the European Academy of Paediatric Dentistry (EAPD) 2003 seminar held in Athens [2]. If a tooth had more than one hypomineralised lesion, the largest and most severe lesion was used to classify the tooth. The following definitions were used to classify the teeth:

Demarcated opacity: a demarcated defect, variable in degree, involving an alteration in the translucency of the enamel. The defective enamel is of normal thickness with a smooth surface and can be white, yellow or brown in colour.

Posteruptive enamel breakdown: a defect with loss of initially formed surface enamel, after eruption of the tooth. The loss is often associated with a pre-existing demarcated opacity.

Atypical restoration: in most cases in molars there will be restorations extending to the buccal or palatal smooth surface, or atypical approximal surface (the lack of an adjacent tooth touching the contact point should arouse suspicion as to the cause of breakdown and caries at an approximal site). At the border of the restorations frequently an opacity can be noticed. In incisors a labial restoration maybe present but unrelated to trauma.

Extracted molar due to MIH: absence of a first permanent molar should be compared to the other teeth in the mouth. The clinical findings that lead to suspicion for extraction due to MIH are opacities and/or atypical restorations in the other first permanent molars, or the presence of hypomineralised defects on anterior teeth.

Unerupted: the first permanent molar or the incisor to be examined are not yet erupted.

Each tooth was assigned to one of two categories, mild or severe MIH, based on the clinical findings. Mild MIH was defined as a molar or incisor with no enamel breakdown, but with yellow, brown or white demarcated opacities. In contrast, severe MIH was defined as either post-eruptive enamel breakdown, atypical restorations, or previous extraction due to MIH. Additionally, once the teeth were categorized as mild or severe, each subject was assigned to either mild or severe MIH, based on the most severe classification of their individual teeth. For example, if a subject had one molar with severe MIH and the remainder had mild MIH, they would be considered a subject with severe MIH (Figs. 1,2).

Two dental clinicians (KM, Paediatric Dental Resident; and HK, Paediatric Dentist) at Boston Children's Hospital performed the examinations. The two clinicians, KM and HK were calibrated using intraoral photographs of teeth with MIH.

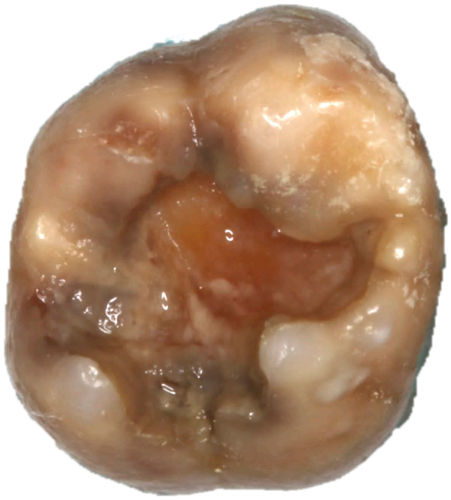

FIGURE 1. An example of severe form of MIH.

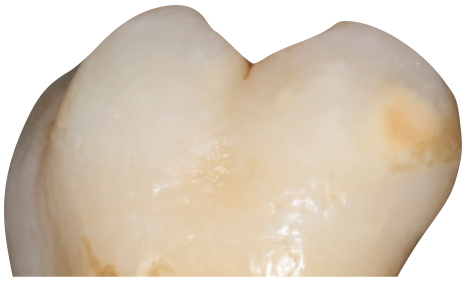

FIGURE 2. An example of mild form of MIH.

The photographs were displayed on a computer in full-screen mode and scored by both paediatric dentists independently at least two weeks apart to reduce recall bias. Inter-examiner calibration was conducted on patients at the start of the project. To test the inter- and intra-observer agreement, Cohen's Kappa was calculated. The formula used for sample size calculation was, $n = [Z2P(1–P)]/d^2$ where n is the sample size, Z is the statistic corresponding to level of confidence, P is expected prevalence and d is precision [26]. Sample size was calculated based on 2.4% prevalence [27] of MIH with 95% confidence interval and precision of 0.05.

In addition to the clinical examination, data was collected through a medical and dental history questionnaire. The information recorded included prematurity, maternal perinatal medical history (fever, illness, medication intake, vitamin D deficiency, maternal age), child's birth weight, gastrointestinal issues, pneumonia, asthma and other respiratory diseases, childhood diseases (chickenpox, rubella, mumps, scarlet fever, measles, hypoxia, neonatal hypocalcemia prior to age 4), long-term breast feeding, frequent fever, febrile convulsions, otitis media, urinary and respiratory infections, fluoride and calcium intake prior to age four, history and dose of penicillin or other antibiotic therapy, age and gender, history of permanent tooth extraction, sensitivity when brushing teeth, aesthetic concerns with regards to incisors and spontaneous/persistent hypersensitivity in the molars.

3. Results

This pilot study included 63 healthy control subjects (29 females, 34 males) and 18 subjects (12 females, 6 males) with SCD. The greatest proportion of patients in both control and SCD groups were of African American ethnicity, 28.5% and 72.2% respectively. The inter- and intra-observer agreements yielded the following Cohen's Kappa scores for MIH 0.85 (inter), 0.90 (intra KM) and 0.95 (intra HK).

Molar-Incisor Hypomineralisation was present in four (22.2%) SCD patients and 24 (38%) control subjects. Of the four SCD patients with MIH, 50% were severe. In comparison, of the 24 control subjects who had MIH, only five (21%) had severe MIH (Table 1). The tooth seen most frequently affected by MIH was the maxillary right first permanent molar, followed by the maxillary and mandibular left first permanent molars. The overall (for SCD and control groups) prevalence of MIH was 34.5% (28/81), with 8.6% (7/81) of these being severe.

TABLE 1. Presence and severity of MIH in SCD when compared with the control group.

MIH	Control (%)	SCD (%)
Presence	24 (38.0)	4 (22.0)
Severity—mild	19 (79.1)	2 (50.0)
Severity—severe	5 (20.8)	2 (50.0)

MIH: Molar-Incisor Hypomineralisation; SCD: Sickle Cell Disease.

In the control group, out of the 24 subjects with MIH, 41.6% (n = 10/24), had a history of antibiotic therapy (<2 weeks) prior to three years of age. In the same group with MIH, 40% (n = 14/24) reported no history of antibiotic use. Interestingly, in the control group, 79.1% (n = 19) of the subjects were diagnosed with mild MIH, five of those subjects a history of antibiotic use. Severe MIH was observed in 20.8% of the control subjects (n = 5) (Fig. 3).

The health history questionnaire demonstrated no statistically significant correlation between MIH and the following: pneumonia, asthma, breast milk, fever, otitis media, flu and upper respiratory infection (Table 2). There was a significantly higher ($p = 0.021$) proportion of severe MIH in subjects with premature birth (21.4%; n = 3/14) compared to subjects with full term birth (5.9%; n = 4/67) (Fig. 4).

There was a significant difference in the presence of MIH in subjects with a history of penicillin-based antibiotic therapy in the control group (n = 10/28, 35.7%) ($p = 0.011$). There were no cases of severe MIH in the non-antibiotic use subset of the control group.

4. Discussion

There is debate in the current literature regarding the relationship of antibiotic use and MIH. Some laboratory studies have found a statistically significant correlation between amoxicillin and enamel thickness in animal models [16, 28–30]. Some clinical and *in-vivo* studies have also shown an association

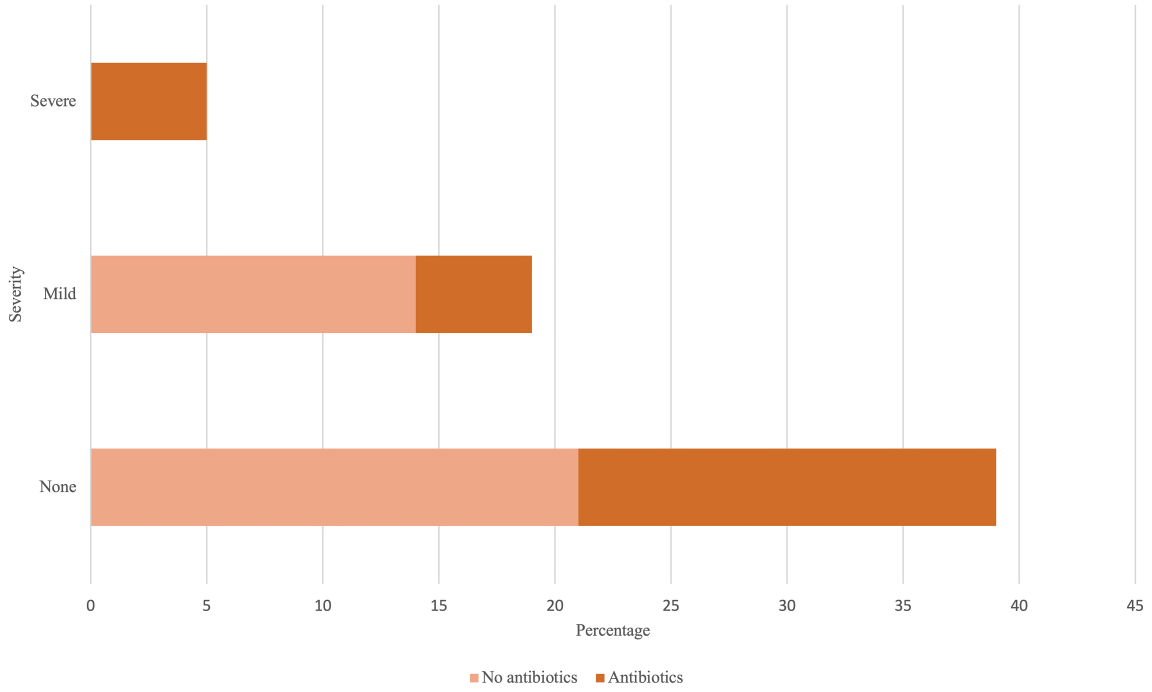

FIGURE 3. The severity of MIH in control population with or without antibiotic therapy.

TABLE 2. Illness-based categorisation of patients with SCD and control group.

Variables	Control—MIH yes (%)	Control—MIH no (%)	SCD—MIH yes (%)	SCD—MIH no (%)
Pneumonia	1 (4.1)	3 (7.6)	0	4 (28.5)
Asthma	5 (20.8)	5 (12.8)	1 (25.0)	3 (21.4)
Breastmilk >1 yr	4 (16.6)	9 (23.0)	1 (25.0)	1 (7.1)
15 mon >1 illness with fever	6 (25.0)	6 (15.4)	1 (25.0)	6 (42.8)
Otitis media	12 (50.0)	12 (30.7)	0	4 (28.5)
Colds/flu	13 (54.0)	20 (51.2)	0	8 (57.1)
Antibiotics use	10 (35.7)	18 (64.2)	4 (22.2)	14 (77.7)

MIH: Molar-Incisor Hypomineralisation; SCD: Sickle Cell Disease.

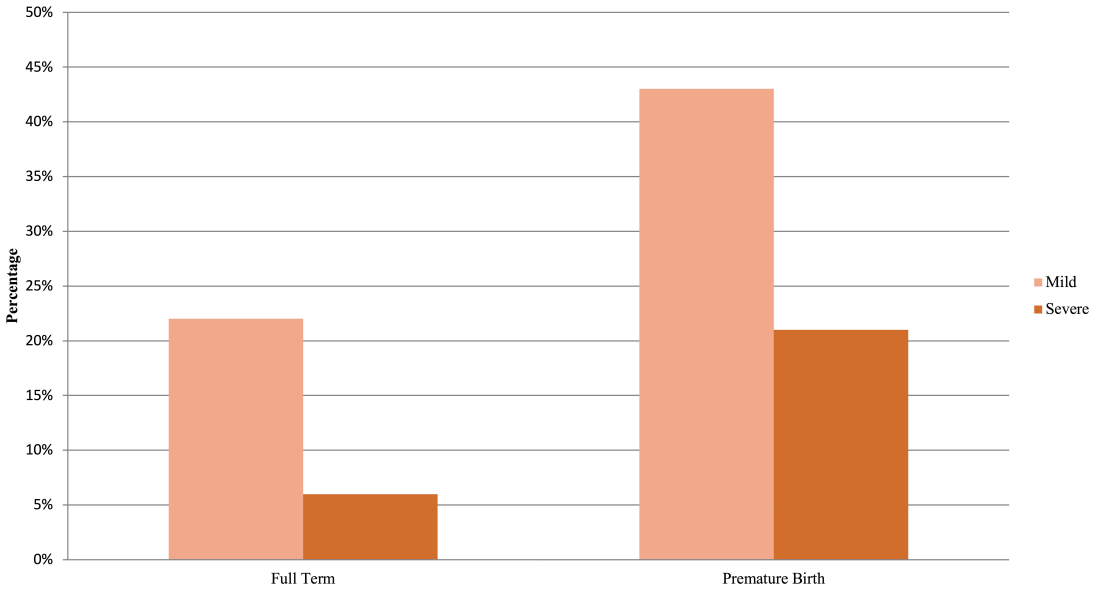

FIGURE 4. The relationship of severity of MIH to premature birth.

between antibiotic use and increased rates of MIH [15–18]. As such, one would expect that patients with SCD, on long-term antibiotic therapy, would have higher rates of MIH than a healthy population of children who were not exposed to antibiotics regularly. Interestingly, our results did not confirm this.

In our study, the prevalence of MIH in the SCD and control group was 22.2% and 35% respectively. This indicated that the majority of the subjects (SCD and control) with a history of antibiotic use had no MIH. However, in both groups, severe MIH was only noted in patients with a history of amoxicillin/penicillin use ($p = 0.011$). Therefore, it could be suggested that the use of antibiotics can increase the risk of severe MIH in a patient predisposed to this condition. This is further demonstrated by the data in the SCD population, as 50% of the cases of MIH were severe. Therefore, it could be argued that a patient with SCD who presents with MIH is likely to be predisposed to having a severe form of MIH. Further larger scale studies are required to investigate this finding.

Current literature supports the correlation between developmental defects of enamel and pre-term birth [31–33]. Our study confirms that those who were born prematurely were more likely to have MIH ($p = 0.021$). This is likely due to a disturbance in enamel formation of the first permanent molars and incisors at or around the time of birth [34].

It is important for clinicians to be aware of MIH, as their ability to counsel patients on its presence and to encourage timely pre-emptive preventative programs could be crucial in preventing further post-eruptive breakdown in affected molars and incisors. Furthermore, MIH can be a costly diagnosis for a patient, resulting in a lifetime of endodontic therapy and restorative care. Molar-Incisor hypomineralisation can pose challenges for restorative treatment, such as poor bonding, marginal breakdown, frequent failures, difficult anaesthesia and may result in increased cost, time and repeated treatment [3, 36]. If the incisors are involved, the poor aesthetics and associated psychosocial effects on the child can complicate the restorative challenge [35]. Veneers or full coverage restorations may be required to help improve aesthetics [36].

Overall, this study found an MIH prevalence of 35% (including both control and SCD groups), which is higher than the published global prevalence [6]. Additionally, the prevalence of MIH in the control group was 38%. This is likely due to the non-representative nature of the sample and due to the low sample size. The study was conducted at a specialist tertiary referral centre, where complex cases such as difficult to restore hypomineralised teeth are referred for treatment and therefore, cannot be considered a generic representation of the US population.

However, our study results were similar to the South Texas Oral Health Network study [9] which assessed 1212 patients between 6–14 years of age, reporting an overall prevalence of MIH of 29.5%. This cross-sectional research network study was the first report of MIH in the USA [9]. In contrast, Davenport and colleagues reported a 9.6% prevalence in Milwaukee [7]. Similarly, Hartstock and colleagues reported a significantly lower rate of MH [8]. The differences in prevalence rates is likely due to multiple factors including sample bias, limited sample size and epidemiological tools used to assess MIH. Therefore, further standardised epidemiological research is required to determine the range of prevalence rates in the USA.

This pilot study is the first of its kind assessing the relationship of MIH in patients on long-term antibiotic therapy. Some of the limitations of this study include a small sample size, difficulty controlling all variables and recall bias. Many generalisations are made based on a small number of participants. Accordingly, most of the associations seen in this study are statistically significant but many would be worth delving into further. Therefore, it should be used as a guide to implementation of further research.

5. Conclusions

This study demonstrates no significant correlation between long-term antibiotic use and MIH in patients with SCD patients. However, the use of antibiotics, especially penicillin-based, may increase the risk of severe MIH in patients with SCD. It also demonstrates a link between pre-term birth and MIH. MIH was not associated with a history of pneumonia, asthma, breast milk, fever, otitis media, flu or upper respiratory infection.

AVAILABILITY OF DATA AND MATERIALS

The data are contained within this article.

AUTHOR CONTRIBUTIONS

HK and IC—conceived the ideas. HK and KM—collected the data. HK and KM—analysed the data. CN and HK—led the writing.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This prospective cohort pilot study, was approved by the Boston Children's Hospital Institutional Review Board (IRB-P00012054). Informed consent was obtained from the legal guardian and informed assent obtained from each research subject.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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