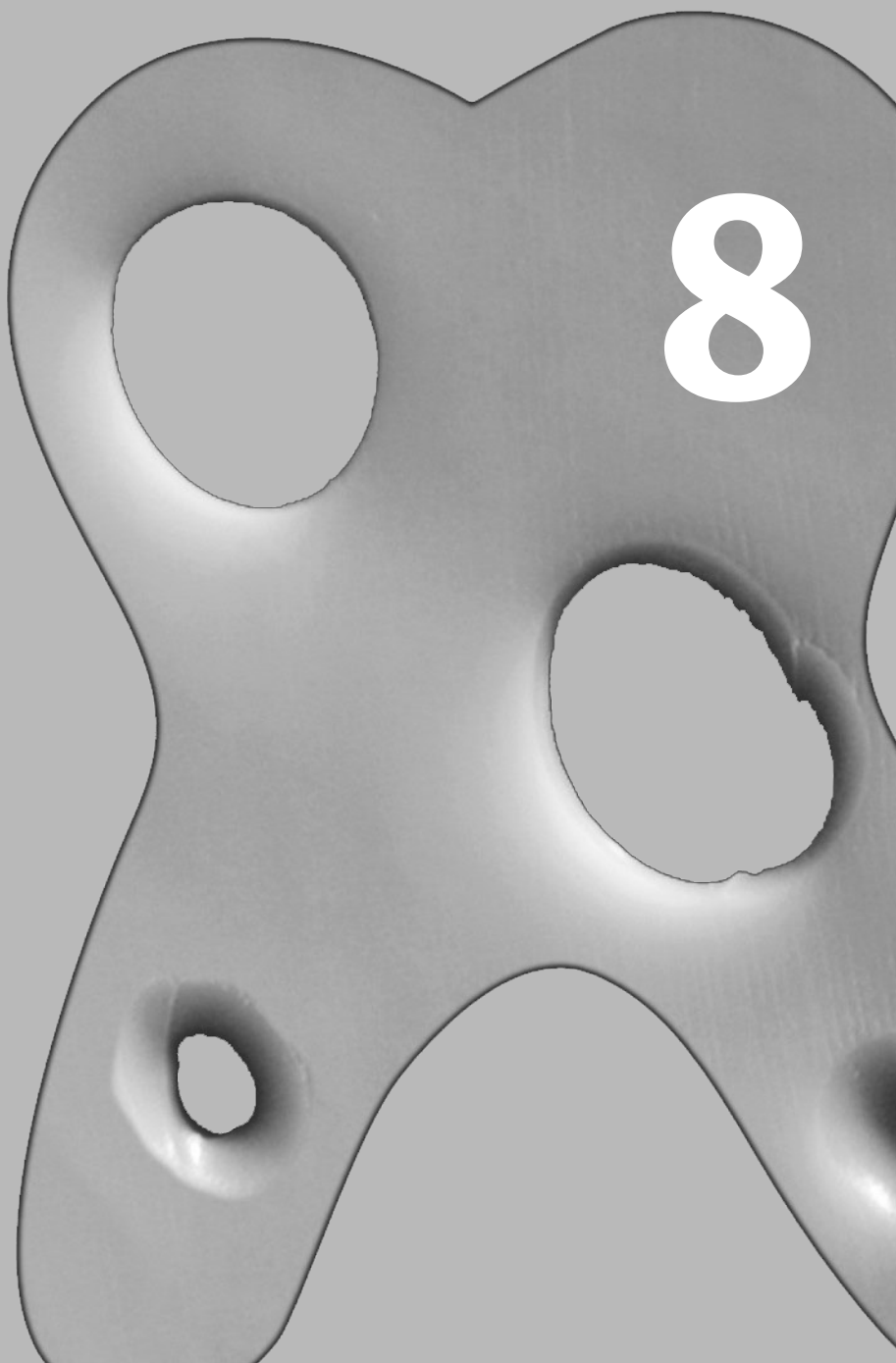
General discussion and clinical implications



Molar Incisor Hypomineralisation (MIH), the hypomineralisation defect in the permanent dentition, has been studied for many years. In 2003, Weerheijm et al. (1) were the first to state that the hypomineralisation defects described as MIH can also be seen in second primary molars, a process later known as Deciduous Molar Hypomineralisation (DMH). Recently, Jalevik (2) advised that the second primary molars and first permanent canines should also be examined when studying the prevalence of MIH. This thesis describes DMH in various aspects, such as its prevalence, relationships with caries and with Molar Incisor Hypomineralisation (MIH), mineral content in the DMH enamel and possible determinants and associated factors.

Prevalence

In earlier years, DMH and MIH were of unknown influence on the caries prevalence of second primary molars. Weerheijm et al. (1) reported that not only first permanent molars and incisors are affected by hypomineralisation but also the second primary molars. Our study on the prevalence of DMH in 5-year-old Dutch children was the first study on the prevalence of DMH worldwide (3). Most studies on hypomineralisation in the primary dentition reported all primary teeth. Looking only at the second primary molars, as we did, will automatically give a lower prevalence at child level. In our studies DMH was diagnosed with the same criteria as used for MIH, which makes it possible to make a comparison: the prevalence of DMH is lower than the prevalence of MIH in Dutch children (9.7%-14.3%) (4, 5). The prevalence of DMH in the Generation R study was higher than in the TJZ study (9.0% vs 4.9%) (3, 6) and it was more in line with the prevalence of MIH in other studies in the Netherlands. Because the children in both studies were around the same age and the same scoring criteria were used, age differences and scoring differences can not be an explanation. Probably the fact that Rotterdam is a large multicultural city and the TJZ study comprised smaller cities could be one of the explanations. The exposure to possible determinants could also be an explanation, but data on the pre- and perinatal period of the children in the TJZ study are not available.

From MIH research we also know that prevalence can differ between consecutive birth years without a clear cause (2). This unexplained variability could also be occurring in DMH, and additional research, for example in the TJZ study, is needed to examine this possibility.

Relationship between DMH and caries

The two studies described in chapter 4 showed that second primary molars are affected more by caries than first primary molars (7, 8). Many studies had previously been done on the description of caries patterns and the prediction of caries (9). The first primary molar could be assumed to have more caries due to its longer presence in the oral cavity, but this theory is not supported in the literature. Only in special cases (e.g., early childhood caries) are teeth attacked by caries in the sequence of eruption (10, 11). The difference in caries can be explained by the hypomineralisation of second primary molars. In our study, DMH was shown to be an important explanation, next

to ethnicity, for the differences in caries found between the first and second primary molars (8). When DMH is the main reason for the caries differences, the hypomineralised areas would be found in the most vulnerable parts of the teeth. The differences in caries on the occlusal surface between first and second primary molars were the most pronounced in our research. Most opacities were also found in the occlusal third of the molar, which is in line with opacities being more prone to caries.

Mineral content of DMH molars

In chapter 5, the mineral (hydroxyapatite) density of sound and opaque areas was determined in both DMH and unaffected molars. The reductions in mineral density of clinically unaffected enamel and in yellow opacities were similar to those reported for white spot lesions (12). This structural weakness could lead to an increased sensitivity to cold, warmth and sweets, and the teeth will be more vulnerable to the development of caries and posteruptive breakdown, similar to MIH molars. The mineral content of the yellow opaque areas in DMH molars is in between that of dentin and sound enamel. The hypomineralised enamel does not contain collagen fibres as does dentine, and therefore the structure of the hypomineralised areas could be even weaker. We suggest that the treatment of hypomineralisations should follow the same protocol as the treatment of demineralisation: regular applications of fluoride or Casein PhosphoPeptides and Amorphous Calcium Phosphate (CPP-ACP) to stimulate remineralisation of the enamel (13, 14). This treatment will protect the teeth against caries and decrease their sensitivity to warmth, cold and sweets.

Determinants and associated factors of DMH

In a prospective cohort study, the determinants of DMH were described. In this study, ethnicity, low birth weight, alcohol consumption by the mother during pregnancy and fever of the child in its first year of life were found to be determinants for DMH. The relationship between the use of either antibiotics or asthma medication during pregnancy and DMH was also studied, but no associations between medication and DMH were found. More research is needed to determine the principles behind the relationships that were identified.

Ethnicity, as found in this study, can be an indication of the involvement of a genetic factor. Most studies on MIH and DMH are performed in Europe, probably because it is most often seen here. This suggests that the Caucasian background might be related to a lower threshold.

For MIH, low birth weight did not appear to be a determinant. Low birth weight could be associated with DMH, but caution needs to be exercised because the research from Vello et al. (15) and Rugg-Gunn et al. (16) examined the enamel defects on all primary teeth and used a different index (mDDE) to score the enamel defects. Moreover, low birth weight is a variable that involves a significant number of co-morbidities.

Based on animal research, ethanol (alcohol) has been shown to lead to changes in, for example, cellular differentiation and enamel mineralisation (17). An association of alcohol use with hypomineralisation in humans has not been previously reported. In our study we found that alcohol consumption of the mother during pregnancy is one of the determinants of DMH.
Fever is often mentioned in MIH research (18) as a possible aetiological factor. In MIH research at the moment, whether the disease, its co-morbidity, the fever that usually comes with the disease, or the medication used to treat the disease (antibiotics) causes the hypomineralisation is unknown. Several diseases were found to be associated with MIH, including otitis media, pneumonia, asthma, urinary tract infections and chickenpox, although controversial results were sometimes found (18-22). Most likely, the disease itself does not cause the hypomineralisation, but rather high temperature, as a common symptom, or antibiotics, as a common treatment of the abovementioned diseases, is responsible. In an animal study, hypomineralisation of incisors could be induced by fever in rats (23).
The cause of DMH is multifactorial, and some of the same determinants were found as have been proposed for MIH. This overlap indicates a direct relationship between DMH and MIH, which was also shown in the study on the relationship between DMH and MIH.

Relationship between DMH and MIH
In this study (chapter 7), the association between DMH in the second primary molars and MIH in the first permanent molars was described. Children with DMH have a higher risk for MIH than children without DMH (OR: 4.4), but interestingly, children with mild DMH (opacities only) had a higher risk than children with severe DMH (6). The relationship between DMH and MIH is important for clinical practice: extra attention needs to be paid to those children with DMH during the period that their permanent molars and incisors are erupting, given their increased risk of having MIH. Using DMH as predictor for MIH could help with this clinically important early diagnosis. Predicting which teeth might be affected with MIH based on the molars affected with DMH will hopefully be possible in future.

METHODOLOGICAL CONSIDERATIONS

In the previous chapters, specific strengths and limitations have been discussed for the specific studies and their populations. Now, a more general discussion will follow, also comparing the strengths and limitations of the different study populations.

Study design
In this thesis, different study populations were used. The smallest group comprised the children who donated their teeth for the microCT research. These referred children were all treated under general anaesthesia in one of the paediatric dental practices in Amsterdam or Haarlem.

The 62 children in the validation study were also referred children, visiting a dental practice in Amsterdam or Oldenzaal (small city in the eastern part of the Netherlands). Both populations are convenience samples of the population in the dental practice of paediatric dentists. Many children who are referred to a paediatric dentist have caries and/or hypomineralisation defects (DMH and MIH). The prevalence of caries and DMH is therefore higher than in the average Dutch population, and the children should therefore not be considered a random selection that is representative of the population. Outcomes on the prevalence of DMH and caries in these studies should not be compared with the other studies.

The TJZ study was carried out in four medium-sized Dutch cities: Gouda, Alphen aan de Rijn, ´s Hertogenbosch and Breda. The trends seen in these cities are considered to be representative of the trends in the Netherlands (24). The comparison of the TJZ study with the Generation R study and the generalisability of the results for the whole Dutch population should be done with caution.

The children participating in the TJZ study were all insured by Health Insurance Funds.

Insurance by such funds was compulsory for individuals earning less than some income criterion and for their family members, covering altogether approximately 60% of the Dutch population (25). Therefore, the TJZ study included only the lower income groups and in the Generation R study all income-groups were aimed at. Because household income can be a confounder in the research on caries and hypomineralisation, comparison between the Generation R study and the TJZ study has to be done with caution.

Validity and reliability of intra-oral photographs

In chapter 3, the validity and reliability of intra-oral photographs are described. This study proved that the validity (based on the sensitivity and specificity) and reliability (based on Cohen´s kappa scores for inter- and intra-observer agreement) of the camera was high (26). Therefore, the camera could be used in the Generation R study. Of course there are also some drawbacks in using an intra-oral camera specifically for a large epidemiological study. Because the lifetime of the camera was less than the duration of the epidemiological study, cameras needed to be replaced. Differences between the photographs made with different types of cameras were checked and no differences in diagnosing DMH and caries were found. The major difference was the colour tone of the photographs. Due to these colour differences, the visualisation of tooth-coloured restorations was more difficult, and the colour of the opacities could not be judged reliably.

One of the major advantages of taking photographs of the teeth is that the photographs can be stored and used in a later phase of study for comparisons or additional research, such as to assess inter-observer reliability.

Selection bias

In the validation study and microCT study, all children who were asked to participate agreed. Because these samples were convenience samples, the outcome cannot be generalised. We decided to make a descriptive analysis of the differences in the mineral contents of the DMH molars with and without opacities using healthy molars as a control. Future studies will need to confirm the clinical consequences of hypomineralisation.

The TJZ study, needed for the epidemiological data, had a participation rate of 63% in 1999 and 38% in 2005. In a group of non-participants, the reasons for non-participation were asked using a short questionnaire; the answers revealed that there were no statistically significant differences between participating and non-participating children with respect to tooth brushing frequency, education level of the mother, and country of birth of mother and child. The group participants were therefore considered to be representative.

In Rotterdam, 61% of the eligible children participated in the Generation R study (27). In the Generation R study, mothers of lower socio-economic status and from ethnic minorities and mothers and children with medical problems seemed to be underrepresented. This underrepresentation would imply that the study population was healthier and had a higher social economic status (28).

Most data in the Generation R study were collected via questionnaires. To also reach parents from ethnic minorities, questionnaires were also available in English and Turkish. For other languages, interpreters were available for support. Despite these efforts, participants who did not return their questionnaires were most often not of Dutch origin and were less educated, younger or had a less healthy lifestyle (28).

At the age of 5 or 6, 6690 children visited the research centre, and most of them had photographs taken of their teeth. The ethnicity and education level of the mothers and children participating in this part of the study showed that Dutch mothers and mothers with higher education more often participated in this research.

Information bias

The TJZ and Generation R studies both used questionnaires to collect data on factors including socio-economic status, ethnicity, health and behaviour. These questionnaires were based on earlier validated questionnaires.

Data collected with questionnaires reported by the parents are generally believed as being less reliable because people tend to give socially desirable answers (29). Questionnaires also depend on the memory of the parents as questionnaires typically ask for situations and conditions in the past (30); details pertaining to last month are much more reliable than those of years ago. So, the frequent questionnaires in the Generation R study during pregnancy and the first year of life of the children should have provided a good picture of the possible medical and environmental factors and overcome some of the drawbacks of research with questionnaires.

FUTURE PERSPECTIVES

Prevalence

Because our study was the first to report on the prevalence of DMH, prevalence studies in other countries also need to be performed, by preference embedded in the MIH research. As ethnicity is found to be a determinant for DMH, the differences in prevalence between different countries and continents will be interesting.

Most of the MIH research has been done in northern Europe (Sweden, Finland and the Netherlands), and the prevalence found in those countries seemed to be higher than in other countries. The same will likely be the case for DMH.

Mineral density

The enamel in DMH molars has a lower mineral content, approximately the same as in white spot lesions (12). Hypomineralisation of the second primary molar is the main reason for the caries difference between first and second primary molars.

In vitro and in vivo studies should be performed to see which preventive measures (e.g., application of fluoride or CPP-ACP, or micro-invasive infiltration therapy (ICON)) can be used for DMH molars to prevent them from getting carious or from posteruptive breakdown. The sensitivity of the DMH teeth could likely be treated in the same way.

Determinants and associated factors

This study is only the start of elucidating the determinants of DMH. In the literature, many different factors influencing tooth development have been studied. These studies are typically animal studies, investigating a specific relationship between one factor and its influence on amelogenesis. Factors such as dioxins (31), medication (32, 33), fluoride (34) and temperature/fever (23, 35) were found to cause disturbances in amelogenesis. From these studies, the tissues involved in tooth formation could be concluded to be very sensitive during the period of amelogenesis. Since we found an association between DMH and MIH, comparable determinants must play a role. The children participating in the Generation R study will be invited to visit the research centre again at age 9. At that age, most children have all their first permanent molars and incisors erupted, so MIH can be diagnosed. Those data, together with the data collected on DMH in the same children at the ages of 5-6, will provide better insight into the determinants and their interactions involved in hypomineralisations.

Genetic factors are likely to be also associated with DMH. Genetic variation could possibly explain the differences between individuals with the same exposure to risk factors. In the Generation R study, Single Nucleotide Polymorphism (SNP) data are available. To perform a Genome Wide Association (GWA) study, data from the children in Rotterdam need to be compared with data from a large group of children from another setting. Genes influencing tooth development have

been studied (36), but no studies were done for DMH or MIH. A GWA study in Caucasian children can bring more insight into the genetic factors of hypomineralisation.
The genome of individuals from different races is different and the genome is continuously adapting to local environments. The genome is likely to be influenced also by infectious diseases and its pathogens (37). Probably also medical care influences the genome by opposing the natural selection. As a consequence, the factors influencing the genome can give a less healthy genome for hypomineralisations in the Caucasian children.
In recent years, epigenetics, the change in gene expression without changes in the DNA, has received attention in the medical world, especially in cancer research (38). DNA methylation, which regulates genetic expression and integrity in various biological processes such as cell differentiation, is important in epigenetics. The areas where this DNA methylation can occur are not equally distributed along the DNA strand but tend to be clustered in so-called CpG-islands. The genome methylation pattern is inherited during mitosis and is tissue-specific. If a gene is methylated in its promoter region, the gene will not, or only at a low rate, be transcribed into messenger RNA (mRNA), thereby reducing the expression of that gene (38). DNA methylation can be influenced by excessive or deficient nutrient status. Amongst others, folate, vitamin B6, vitamin B12, vitamin A, alcohol, zinc and selenium are known to influence DNA methylation. Environmental toxins seem to affect epigenetic pathways mainly in the same way as nutritional factors and should be studied simultaneously (38).
Because alcohol was identified as a determinant and nutritional factors and environmental toxins have also been mentioned as determinants for MIH (18, 39), their influence needs to be further studied taken epigenetics into account.
Probably not the pollution of the environment but the human reaction on this is the cause of DMH and MIH. If this is true, epigenetics are more important than natural selection for the predisposition of hypomineralisation. As a consequence, DMH and MIH will be more prevalent in future.

CLINICAL IMPLICATIONS

Because the relatively high prevalence of DMH and its relationship with MIH, clinical treatment is important. Children with MIH need more dental treatment and are - as a consequence - more fearful for dental treatment (40). The same could be expected for children with DMH. Early diagnosis of DMH is important, since regular application of fluoride or CPP-ACP can help in remineralising the enamel and prevent posteruptive enamel loss. Because the hypomineralised teeth develop caries more easily, regular dental check-ups are advised on. Around the age of 6, when the first permanent molars will erupt, the check-ups need to be more frequent for early diagnosing of MIH.

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