Molar-incisor hypomineralization (MIH) in a group of school-aged children in Benghazi, Libya

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Abstract

Aim: Molar-incisor hypomineralization (MIH) is common in many countries and it has a significant impact on treatment need. The aim of the present study was to assess developmental enamel defects with an emphasis to MIH in children from four primary schools in Benghazi, Libya. **Methods:** Permanent first molars, totaling 378 (188 in females) in 7.0-8.9-year-old children were examined for demarcated opacities, diffuse opacities and hypoplasia in their schools using a portable light, a mirror, and a probe. A subgroup of children attending two of the four schools and having all incisors and first molars erupted (N = 154) was examined for enamel defects in these teeth. **Results:** There were 11 children (2.9%) presenting with MIH. The mean value of demarcated opacities in their first molars was 1.5. MIH lesions were found only in 1.1% of the children's first molars (tooth prevalence) and all lesions were mild. Six children (1.6%) had diffuse opacities and 3 (0.8%) had hypoplastic defects in their first molars. Fourteen out of 154 children (9%), who had both incisors and molars examined, had some kind of developmental enamel defect: 11 children (7.1%) had demarcated opacities, 3 (1.9%) had diffuse opacities, and none had hypoplasia. **Conclusion:** MIH was rare in Benghazi, Libya. The prevalence was clearly lower than in comparable studies performed in Italy or in Nordic countries, where, according to the earlier reports, MIH is seen in every fifth or sixth child. Our result may be valuable when so far mostly unknown etiology behind MIH is investigated.

Introduction

Molar-Incisor Hypomineralization (MIH) can be defined as a clinical condition characterized by dental enamel defects affecting one to four of the permanent first molars and frequently the incisors [Weerheijm et al, 2001]. MIH can appear as an abnormal demarcated translucency of the enamel with the colour ranging from whitish creamy to yellowish brown. In its severe form secondary breakdown of the enamel is seen. In the literature, MIH is also known as non-fluoride enamel opacities, internal enamel hypoplasia, and idiopathic enamel opacities in the permanent first molars [Koch et al., 1987]. A recently published questionnaire assessment on MIH suggested that MIH is widely known in European countries and is considered a clinical problem [Weerheijm and Mejare, 2003]. Its clinical impact has also been shown in a Finnish study where, in a group of children with MIH, the DMFT values in 7-13-year-old children were more than twice as high as in the control group [Leppäniemi et al., 2001].

Available prevalence data of MIH in European countries vary from 3.6 to 25% with the highest percentages in Nordic countries [Koch et al., 1987; Esmark and Simonsen, 1995; Alaluusua et al., 1996a,b; Leppäniemi et al., 2001; Weerheijm et al., 2001; Jälevik et al., 2001a; Hölttä et al., 2001; Dietrich et al., 2003; Calderara et al., 2005]. Part of the wide prevalence range may be explained by differences in diagnostic criteria and selection of the study populations. However, substantial data suggests that children in certain regions and birth cohorts have more MIH than in others [Koch et al., 1987; Hölttä et al., 2001; Dietrich et al., 2003].

An exact aetiology of MIH has not yet been established but it is generally agreed that MIH is caused by a systemic factor. In the first epidemiological studies on hypomineralized permanent first molars and incisors, Koch and coworkers [1987] suggested that some environmental factors may be behind these defects. This was based on their finding that Swedish children born in 1970 had more defects than those who were born earlier or later. Effects of environmental toxicants on developing teeth have been studied in Finland and the results suggest that children exposed to higher amounts of dioxins via mother's milk have more often presented with MIH than children who are less exposed [Alaluusua et al., 1996b].

Key words: Enamel opacities, Enamel hypomineralization, teeth, children.

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Oxygen shortage at the time of delivery and respiratory disorders during the first three years was suggested to be important factors behind MIH in a Dutch study [Van Amerongen and Kreulen, 1995]. In another Dutch study it was suggested that affected children may have suffered more episodes of illnesses such as otitis media or high fever [Beentjes et al., 2002] and in a Swedish study, MIH was associated with respiratory diseases in childhood [Jälevik et al., 2001b]. A role for antibiotics has also been suggested [Jälevik et al., 2001b; Beentjes et al., 2002] but also opposing results have been found [Tapias-Ledesma et al., 2003].

In the present study, an evaluation of the prevalence of MIH in a group of school-children living in Benghazi, Libya, in relation to other developmental defects of enamel in permanent first molars and incisors, was carried out. No published data on developmental defects of enamel concerning the present area or Libya was available. The aim was to obtain comparable prevalence data on MIH.

Materials and methods

Population selection. Up to 400 children (200 females) at their second or third class from four different primary schools were screened for developmental defects of enamel in the permanent first molars. Of these children 22 were excluded as none of the permanent first molars (hereafter called molars) had erupted yet. A subgroup of children attending two of the four schools and having all incisors and first molars erupted (N = 154) was examined for enamel defects in these teeth.

The children lived in Benghazi which is considered as the second largest city after the capital, Tripoli. It has about a million inhabitants living mainly on the Mediterranean coast. Benghazi consists of three main districts and our study included four primary schools in Benghazi Eljadeda district-Alfwehat region. Flouride level in the tap water is 0.40 mg/L in the region. The socio-economical status of the population of this region is generally considered the highest among the inhabitant of Benghazi.

Examination criteria. Special emphasis was put on MIH and the following criteria were used. Each molar and incisor was examined for the presence and severity of demarcated opacities. Accordingly, colour changes (white/opaque, yellow or brown) of smooth tooth surface were considered as mild defects. Loss of enamel without dentine involvement was considered as moderate defect and lesions affecting both the enamel and the dentine, atypical restorations replacing affected hard tissues and teeth extracted because of severe hypomineralization were considered as having severe defects.

Teeth with less than half of their crown erupted were excluded and only lesions which were 2 mm in diameter or larger were included [Calderara et al., 2005]. A modified DDE Index was used in the evaluation of diffuse opacities and hypoplasia [Clarkson and O'Mullane, 1989]. Hypoplasia and posteruptive enamel breakdown was distinguished by means of visual inspection of the borders of the defect. Round and smooth borders indicated hypoplasia, while more irregular and sharp edges indicated posteruptive enamel loss [Weerheijm et al, 2001].

One dentist (DF), assisted by another dentist (AA), who was trained for the study in Finland, performed the dental examination in daylight using a torch, dental mirror, and dental probe whenever necessary. Teeth were dried slightly with gauze before registering enamel defects. The examination took place in the pupils' classrooms while they were sitting on their ordinary class desks.

Reproducibility. The reproducibility of diagnosing developmental defects of enamel was assessed by examining 50 children on two occasions. Intra-examiner kappa was 0.84.

Statistical analysis. Chi-square test was used to compare the presence of demarcated opacities in molars and incisors. A probability value of less than 0.05 was regarded as significant.

Results

Population. The children examined were born in 1996 and 1997, the age range varying from 7.0 to 8.9 years (mean of 7.8 years). Altogether, 378 children (188 females) were eligible, 369 having all molars erupted, 4 having 3 and 5 having 2 molars erupted.

Prevalence of MIH. There were 11 children (2.9%) with MIH and the condition was nearly equally distributed among boys (N = 5) and girls (N = 6). All lesions were mild. Only one molar was affected in 7 children, 2 molars in 3 children and all 4 molars in one child. The mean value of demarcated opacities in molars of children with MIH was 1.5. Considering tooth prevalence, demarcated opacities were found only in 1.1% of the children's molars. Six children (1.6%) had diffuse opacities and 3 (0.8%) had hypoplasia in their molars. The numbers of affected teeth were: 17 molars with demarcated opacities, 15 with diffuse opacities and 3 with hypoplasia.

In the subgroup of children with all molars and incisors erupted (N = 154) 14 (9%) had some kind of developmental enamel defects. The total number of defective teeth was 50 (2.7%), 18 (36%) being molars and 32 (64%) incisors. There were 11 children (7.1%) with demarcated opacities, 3 (1.9%) had diffuse opacities and none had hypoplasia. Demarcated opacities were seen in decreasing order in 2.9% of the maxillary incisors, 1.6% and of the molars; 1.0% of the manibular molars and 1.0% of the incisors. There was a significant correlation between the number of demarcated opacities in molars and incisors ($p<0.000$). Five children (2.5%) had demarcated opacities in their incisors only. Four children had demarcated opacities and only one had diffuse opacities in both upper central incisors.

Discussion

In this study, we found that MIH is very rare in a group of 7.0-8.9 years old children living in Benghazi, Libya and born in 1996-1997. Only 11 out of 378 children had MIH. To our knowledge, this is the lowest prevalence of MIH so far reported but figures nearly as low were reported from Dresden, Germany [Dietrich et al., 2003]. As Benghazi is a large city our results are valid only for the present group of children and cannot be generalized for the other regions of the city. However, when evaluated in the light of other earlier results from Europe, the study confirms that there are regional differences in MIH.

In general, prevalence figures of MIH are difficult to compare because of methodological differences. An important factor that has had remarkable effect on prevalence figures has been the inclusion or exclusion of small demarcated opacities. To increase the comparability of the studies, we recently suggested that lesions smaller than 2mm in diameter should not be included [Calderara et al., 2005] as, firstly, this criterion had already been used in many studies on MIH [Alaluusua et al., 1996a,b; Leppäniemi et al., 2001; Jälevik et al., 2001a;] and secondly, it had been earlier found that the reproducibility of small opacities (less than 2mm in diameter) was low [Suckling et al., 1985]. In addition to the present study, we found three epidemiological MIH studies where similar diagnostic criteria had been used: one from Finland [Leppäniemi et al., 2001] and one from Italy, completed by our group [Calderara et al., 2005], and one from Sweden [Jälevik et al., 2001a]. In all these studies unselected populations have been included. As seen in table 1, the prevalence, tooth prevalence and severity scores of MIH are lower in the present children from Benghazi than in any other group of children. In the Finnish and Swedish groups, MIH is even 6 times more common than in the children from Benghazi.

Permanent first molars start to mineralize at birth and incisors somewhat later [Schour and Massler, 1940]. Due to overlapping of the time of development, it is logical that enamel defects are often seen in these teeth concomitantly resulting in significant association between the number of demarcated opacities in molars and incisors. This association was also found in the present study with low MIH prevalence and it suggests a systemic effect behind MIH.

One possibility of lower MIH prevalence could be good resistance related to ethnicity of children living in Benghazi to factors causing hypomineralizations. This is not likely according to the results of an English study which showed that there were no significant differences in prevalence of enamel defects in permanent molars between children of different ethnic groups. The authors concluded that the aetiology is most likely affecting all children [Zagdwon et al., 2002].

In a field study like the present, hypomineralized enamel may be masked by bacterial plaque. Also the lesions may disappear because of caries involvement or restorations. The younger the children the fewer caries lesions in the target teeth they have which could mask hypomineralizations. For this reason we chose to study 7-8.9-year-old children. We included children who had at least one permanent molar erupted, the inclusion criterion which had been used also in earlier studies [Leppäniemi et al., 2001; Jälevik et al., 2001a; Calderara et al., 2005]. Inevitably then, some of the cases with defective but unerupted teeth could have been missed. To avoid their influence on the parameters used, we selected a subgroup of children with all molars and incisors. We found that the prevalence of MIH in the subgroup was 3.8 versus 2.9 in the whole study population showing that young age and developmental stage of the dentition had some but minor effect to the prevalence figures.

In the subgroup children, we also screened dentinal caries (data not shown) in molars and found caries in 17 out of 154 children (11%). This is only slightly more often than in the Italian study [Calderara et al., 2005] where 20 out of 227 children (9%) had caries in their permanent first molars. As both studies and also the Swedish study [Jälevik et al., 2001a] were performed in similar field situations and as there was no major difference in the caries occurrence in the Italian and the present groups we feel that the above mentioned studies are comparable with our study.

We found very few hypoplastic defects in the present children. The result is in agreement with earlier epidemiological studies [Jälevik et al., 2001a; Dietrich et al., 2003; Calderara et al., 2005]. Also diffuse opacities were few. There was no data on fluoride supplements of the children, but excess fluoride was obviously not a problem as only one child had diffuse opacities on the surfaces of both maxillary central incisors.

Recently it was suggested that children who received amoxicillin in their early childhood, especially during the 3rd and 6th months of life had a higher risk of defects resembling fluorosis in their maxillary central incisors [Hong et al., 2005]. Regarding antibiotic use in Benghazi, amoxicillin syrup is the first antibiotic of choice for children between 0-5 years for middle ear infections which are considered to be among the most common infections affecting children in that age (personal communication, Professor A. Elshakmak, the head of the Central Paediatric Hospital, Benghazi). As prescriptions are not compulsory to obtain antibiotics from a pharmacy, their use as a whole is difficult to assess.

In the present population we did not study the aetiological factors behind MIH. We can only speculate that those factors that have been suggested to be associated with MIH in other reports are fewer in the present group of children living in Benghazi than in groups of children examined in Nordic

Table 1 Prevalence and severity of Molar Incisor Hypomineralization (MIH) in children as reported in four comparable epidemiological studies.

Authors	No of children	Age range (years)	Percentage of children with MIH	Percentage of children with severe MIH lesions	Mean number of defective molars in children with MIH
Leppäniemi et al., 2001	488	7-13	19.3	8.4	2.7
Jälevik et al., 2001	516	7.6-8.8	18.4	6.3	2.4
Calderara et al., 2005	227	7.3-8.3	13.7	0.4	1.6
Present study	378	7.0-8.9	2.9	0	1.5

countries or in Italy. However, to support this speculation, we could not get any precise epidemiological data on the prevalence of respiratory diseases, use of antibiotics in children or incidence of oxygen shortage during delivery in Benghazi. Neither do we have any data available on dioxin levels in the environment or in breast milk in Benghazi.

Conclusion

MIH was found to be very rare in a group of children living in Benghazi, Libya. The prevalence figure is clearly lower than those obtained from comparable studies performed in Italy or in Nordic countries, where MIH lesions according to earlier reports are seen in every fifth or sixth child. The result may be of value when so far mostly unknown etiology behind the MIH defects is investigated.

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Pulp cell cultures obtained with two different methods for in vitro cytotoxicity tests

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Abstract

Aim: To describe two different protocols for obtaining primary pulp cell cultures, one derived from explants and the other following dissociation into single cell suspension by enzyme digestion. **Methods:** Human pulp tissue was obtained from three healthy premolars. The harvested pulp tissue was prepared for culture using physical methods (one of the premolars) and enzyme, type XI collagenase, (the two remaining premolars). **Results:** In the case of explant based culture, only limited growth was observed in some cases. However, by enzyme digestion, after two weeks cell growth was evident, and differences in cell type were observed according to the tooth involved. **Conclusion:** It has been possible to obtain abundant biological material using an enzyme digestion-based protocol for testing purposes, with low experimental variability, as all cells originated from the same individual.

Introduction

An increasing number of new materials and clinical procedures are introduced in both dental practice and in other fields that require prior evaluation. In paediatric dentistry, despite important advances in the prevention of caries, many teeth are still prematurely lost. Such early dental losses give rise to malocclusion and esthetic, speech and functional problems. One of the main objectives of paediatric dentistry is to preserve the primary dentition until it is replaced by the permanent teeth. In relation to this point, the pulp tissue has important functions to sustain teeth, providing: 1) nutrient supply for dentin/pulp metabolism to maintain the mechanical properties; 2) innervation and sensory organ function for the prevention of deep caries; 3) reparative dentine formation during pulp wound healing process; and 4) immunological response to bacterial infiltration.Nakashima M., 2005. Therefore, all precautions should be taken to preserve the vitality of the primary pulp. In primary teeth when caries are deep and coronal pulp is affected, pulpotomy has been performed. Formocresol has long been the standard pulpotomy medicament in primary teeth, though in recent years its use has been questioned because of the toxic effects. At present, different alternatives are available, including ferric sulphate and mineral trioxide aggregate [Cortés O et al., 1997; Casas et al., 2004; Holan et al., 2005]. Each agent is considered to represent an alternative but requires the prior conduction of pulp biocompatibility and toxicity studies. As a result, not only clinical studies but also experimental research involving in vivo and in vitro models is important. Animal research poses a number of problems, as a careful experimental design must first be developed, with adequate selection of the animal species and prior approval by an ethics committee. The animals moreover require adequate care, environmental and housing conditions, and the research personnel must be trained in animal management and these studies sometimes comprise a large number of variables such as marginal microleakage or contamination during the procedures carried out [Langeland et al.1969; Eisenmenger and Zetner,1985; Pascon et al,1991].

On the other hand, in vitro methods offer cell cultures as a complement to animal experimentation and in some disciplines, such as genetics and toxicology, they constitute alternatives to research in animals. Cell culturing comprises those techniques designed to keep plant or animal cells, tissues or organs alive in an adequate nutritional medium. Cell cultures are the most widely employed option, based on already established cell lines or the use of cells freshly harvested from tissues (primary cell cultures). In such cultures the tissues are deliberately disaggregated using physical methods (explants) or enzymes that digest the intercellular bonds to yield independent cells that are transferred to bottles containing a nutritive medium [Jakoby and Pastan, 1979; Freshney,1992; Morgan and Darling,1993].

The main advantages of cell cultures includes the use of human cells for experimentation and control of the environmental conditions. Cell cultures are of use in diagnosing diseases, obtaining drug products, developing assisted reproduction techniques and in evaluating the potential toxic effects of chemical substances destined for human consumption [Morgan and Darling, 1993]. For the biological assessment of such substances, the different international scientific commissions establish cell culture-based cytotoxicity testing as the initially indicated technique Stanford, 1980; International Standards Organization, 1992. In this context, it is possible to investigate pulp tissue biocompatibility based on the assessment of cell viability, with the observation of morphological changes and growth patterns. And, in some cases, functionality, mitogenicity and clonal capacity testing may also be indicated.

Key words: Cell culture, pulp fibroblasts, explants, enzyme digestion, cell viability
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