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Molar - Incisor Hypomineralization (MIH) and long - term exposure to 25 - hydroxyvitamin D, measured as Bone Mineral Density (BMD) in adolescents from Northern Norway: an epidemiological study based on the Tromsø study: “Fit Futures”

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Table of contents

Abstract

Aims

The basis of the study was to evaluate if MIH could be related to low levels of vitamin D. Vitamin D is understood to have a key role in both enamel and dentin formation, and maturation, in addition to skeletal integrity. BMD could give a clue of the participants' long - term vitamin D exposure, indirectly giving a reflection of a possible vitamin D impact on enamel mineralization.

Materials and Methods

Data from a cross-sectional health survey among adolescents from Northern Norway in 2010 - 2011, called Fit Futures were used in the present study. All participants born in 1994 with data available from the MIH-study was selected. Only participants with completed blood tests and BMD measurements among these were included in the analyzes. The statistical difference between MIH affected and not affected participants regarding BMD measurements and levels of vitamin D was tested in separate analyses, adjusted for sex, age, height and weight. This was done with SPSS in a logistic regression model.

Results

No statistical difference was found between MIH affected and not affected participants when regards to vitamin D levels and BMD, but both values were lower in MIH affected girls. Boys severely affected by MIH, had on average significantly lower BMD values at the femoral neck compared to boys without MIH ($P < 0.05$). The prevalence of MIH was higher among participants with low serum levels of vitamin D compared to the participants with sufficient levels, but without reaching statistical significance.

Conclusion

Severely MIH affected boys has lower BMD values in the femoral neck, compared to boys without MIH. It seems to be interesting to investigate severely MIH affected individuals in future studies.

Keywords

Molar - Incisor Hypomineralization, Bone Mineral Density and 25 - hydroxyvitamin D

Preface

During my practice time as an odontology student, I met several patients with MIH affected teeth. On most occasions, patients find it embarrassing esthetically, especially if incisors is affected. There are also patients with teeth so severely affected that they have to undergo extraction(s).

Once I assisted a pedodontist extracting four first permanent molars (FPMs) from a boy in full anesthesia, with concerned parents in the waiting room. This is not unusual. Many patients, but especially their parents ask why this mineralization disorder occurs. It is disappointing for us as dental professional not being able to answer this question. With this background, I wanted to delve into the topic MIH.

I would like to thank Andreas, Christer and Guri for all guidance, help and support, before and during the work of this master thesis. It has been an incredibly educational process.

Introduction

MIH

MIH was first described in a Swedish study in 1987 (1). It is defined as hypomineralization of one to four FPMs, frequently associated with affected incisors (2). Other teeth in addition to FPMs and incisors have also been reported to be affected (3, 4).

Clinically, MIH is presented as demarcated lesions, with abnormal enamel translucency. The opacity can appear from creamy - white with hard well - mineralized surface, to yellow - brownish discoloration and enamel disintegration (Figure 1 and 2). In severe cases of MIH, the enamel may undergo Post - Eruptive Breakdown (PEB), facilitating dental caries (2, 5).

In addition to PEB there are several other problems regarding the appearance of MIH. Affected teeth are often sensitive, which in turn can lead to both poor oral hygiene and dental anxiety. Several patients also complain about the esthetics of having MIH, especially when the incisors are affected (6, 7). Treatment of MIH affected teeth range from observation and preventive measures against sensitivity, PEB and caries to restorations and extractions, depending on clinical appearance and the patients symptoms (8). The treatments can be both time - consuming and costly. For the clinician the most obvious clinical problems are to obtain adequate pain control and to select a reliable and sustainable restorative material (9-11).

The prevalence of MIH affected patients ranges in the literature from 2.4 % to 40.2 % (12). Globally the average prevalence reports to be 14.2 % (8), and in 16 years old adolescent in Northern Norway, 13.9 % (3). Clinical appearance of MIH indicates a possible systemic origin (2, 13). However, it is well known that asymmetrical appearance of MIH occur within the same patient, as well as variations in how sever the individual tooth may be affected by MIH. This can be explained by variations in the timely inception of enamel mineralization in homologous teeth (14). The enamel of MIH affected teeth contains less mineral content compared to the enamel in healthy teeth (15). This fact explains from an etiological standpoint the interest for the period of enamel mineralization and maturation of FPMs and incisors, namely the end of gestation to the fourth year of life (7).

The etiology of MIH is unknown. A systematic review from 2016 has assessed the strength of evidence linking etiological factors with MIH (16). They conclude general childhood illnesses like respiratory and high-fever diseases being the most reliable etiological factors of MIH (1, 2, 16). Asthma, allergy, use of antibiotics in young age, prolonged breast feeding, lack of nutrition and minerals are examples of other theories behind the etiology of MIH (16). A genetical relationship of MIH has also been reported (17). A recently randomized clinical trial from 2019, concluded high - dose vitamin D supplementation during pregnancy was associated with approximately 50 % reduced odds of general enamel defects in offspring (18).

The global burden of MIH has been debated among both dental health care personnel and researchers for years. Many dentists and dental hygienists have an impression of an increased incidence of MIH (19). A true increase in the presence of MIH affected teeth is debatable but could be due to environmental factors, differential diagnoses in the past years, raised attention to such teeth, and past - unrecorded cases.

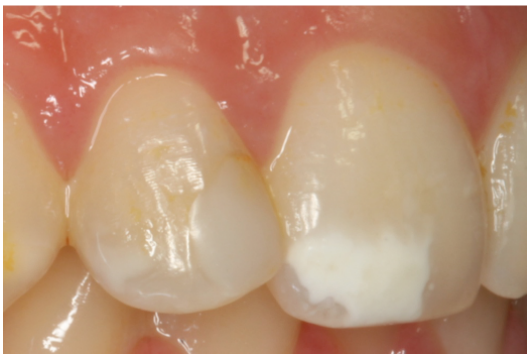

Figure 1 Teeth with opacities from the Fit Futures study (3)

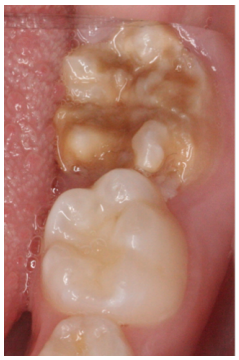

Figure 2 MIH affected FPM from the Fit Futures study (3)

Vitamin D and BMD

Vitamin D is important for many body functions. It has a positive influence on the immune system through induction of defensins and cathelicidin (20). It has in addition an involvement in cell growth, differentiation and immune modulation (21). Vitamin D is understood to have a key role in enamel and dentin formation (12, 22), as well as regulating calcium and phosphorus metabolism and thereby maturation of enamel and skeletal integrity (21).

Vitamin D is produced in the skin when exposed to UVB - radiation (23). Oil rich fish, margarine and some milk products are examples of diet sources, which to a lesser extent enables the absorption of vitamin D (24). The established biomarker of vitamin D, caused by a rapid conversion of vitamin D in the liver, is 25 - hydroxyvitamin D. This biomarker

includes both endogenous synthesis and exogenous intake of vitamin D (25). According to the Norwegian and other European countries guidelines, the sufficient serum level of 25 - hydroxyvitamin D is above 50 nmol/l (26). Could it be plausible that a low serum level of 25 - hydroxyvitamin D have a crucial influence on mineralization and thereby the mineral content of enamel, as seen in MIH?

To reflect on this, it is necessary to know the serum level of 25 - hydroxyvitamin D, during the mineralization period of affected teeth. The teeth cannot be observed clinically during the mineralization period, making it impossible to diagnose MIH in this period. Symptoms of MIH are in most cases not detectable until the age of 8, as it is at this age all FPM and most incisors are erupted (27). From an etiological standpoint, measurement of the serum level of 25 - hydroxyvitamin D is of minor interest when the mineralization period of affected teeth is completed, because of seasonal fluctuations. BMD could be a proxy of the patients long – term, 25 - hydroxyvitamin D exposure, indirectly giving a reflection of vitamin Ds possible impact on enamel mineralization, since bone are remodeling continuously through life (15).

A study published by *van der Tas* et al. (2016*)* examines the association between bone mass and hypomineralized second primary molars (HSPM), as well as MIH in 6 - year - olds (15). In this study, children with lower Bone Area (BA) values were more likely to be affected by HSPM, but not with MIH. However, several studies find an association in regard to caries experience (7, 28) and MIH (29) in patients with low serum levels of 25 - hydroxyvitamin D.

In Northern Norway the prevalence of 25 - hydroxyvitamin D deficiency among adolescents are high. This is probably related to the access to UVB - radiation (26). Several studies conclude an association between caries experience and low serum levels of 25 - hydroxyvitamin D (28, 30). Since caries is associated with low values of 25 - hydroxyvitamin D, one can assume that low levels of 25 - hydroxyvitamin D can lead to less mineralized teeth and further greater susceptibility to caries, and MIH. According to “SSB tannhelsetenesta 2015”, caries prevalence has for years been high among adolescents in Northern compared to Sourthern Norway, which may be thought to be explained by the low levels of 25 - hydroxyvitamin D in Northern Norway, already discussed. Fortunately, the inequality between caries prevalence in the two parts of Norway seems to be decreasing (31).

I hypothesized that MIH could be associated with vitamin D exposure during development and maturation of the enamel, as measured by the proxy BMD in adolescence.

Materials and Methods

Study population and design

The so far most comprehensive health and lifestyle survey among adolescents in Northern Norway is called Fit Futures, and is an expansion of the larger Tromsø study. All first - year upper - secondary school students in the two neighboring municipalities Tromsø and Balsfjord in Northern Norway, were in 2010 - 2011 invited to participate in this cross - sectional health survey for the first time.

Recruitment took place at the schools and information was presented orally in each class. Additionally, a written information brochure was handed out at school and the students were asked to take the brochure home for information to parents and guardians. The information brochure was also available on the school website. Students interested in attending, confirmed it on internet by a link sent to their personal e - mail address and participants signed a written consent for the examination on arrival (26). In order to obtain a high participation rate, the survey was conducted during school hours, and the participants were transported from the schools to the examination stations at the university by mini - buses. Additionally a 200 NOK (35 $ US) bonus check was handed out (31).

The Norwegian Data Protection Authority and The Regional Committee of Medical and Health Research Ethics (REK), (reference number 2009/1282 and 2011/1702/REK nord) approved the study in July 2010 and October 2011, respectively (3, 32). For the present study, three additional approvals were necessary: REK, Norwegian Center for Research Data (NSD), and one for the data - and the publication committee for the Tromsø study (DPU). Due to new legislation, projects that apply for data from the Tromsø study must have their own REK approval. A REK approval was sent and accepted in October 2019, (reference number 31613/REK nord). A notification form to NSD was applied for, to be allowed to process personal data. This was accepted in December 2019 (referencenumber 739051). When these approvals were accepted, the last approval was sent to DPU. Access to the data needed for the present study, was given in March 2020.

Oral clinical examination

The oral part of the survey was conducted by experienced dentists at the University Dental Clinic, The Artic University of Norway. Dental assistants took eight intraoral photographs of each participant (Canon EOS 60D; Canon 105 mm; Sigma EM-140 DG), covering the first and fourth quadrant, second and third quadrant, the buccal surfaces of the maxillary and mandibular anterior teeth, occlusal surfaces of the upper teeth, occlusal surfaces of the lower teeth and the palatinal surfaces of the upper anterior teeth (Figure 3) (3).

MIH was diagnosed based on the intraoral photographs by three experienced dentists on a flat screen in a room with indirect, standardized light. All three dentists studied each participant separately, and scored MIH independently as mild or severe. EAPD guidelines from 2010 was used to score MIH (33). If there was a disagreement, in regards to severity of MIH, among the three dentists, a consensus was reached through discussion. A month later 10 % randomly selected cases with MIH and 10 % without MIH, were re-examined by the same three experienced dentists, in the same way as first time. In the present study only individual born in 1994, with oral clinical photos and without fixed orthodontic appliances were included.

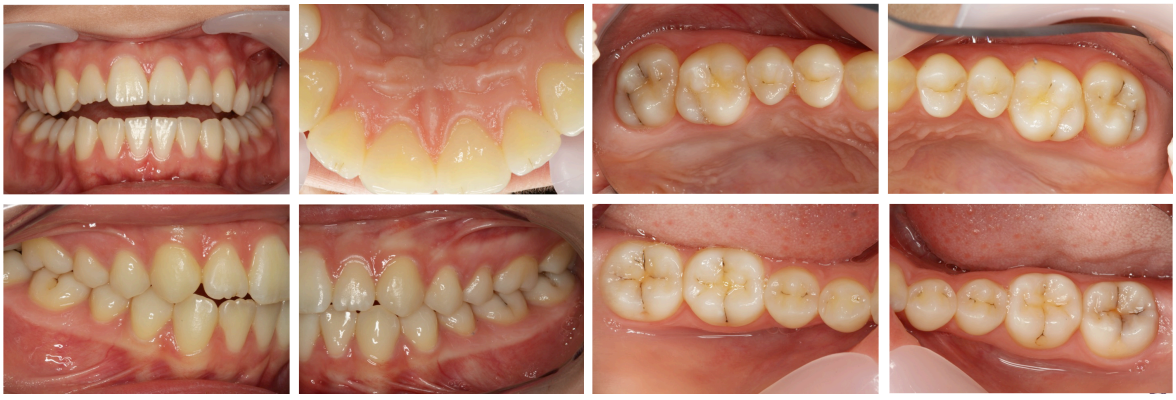

Figure 3 Intra oral photographs from the oral clinical examination from the Fit Futures study of a healthy participant (3)

Clinical examination

All general health examinations were performed in a well - established research unit run by dedicated research technicians at the University Hospital of North Norway (32). In the present study we were interested in only a selection of clinical examination data in addition to the oral examination, this includes BMD and BMI measurements.

BMD

A dual energy X - ray absorptiometry (DXA; GE Lunar prodigy, Lunar Corporation, Madison, WI, USA) was used to measure BMD in all participant (Figure 4). The hip and femoral neck were measured and gave BMD as g/cm^2 (32).

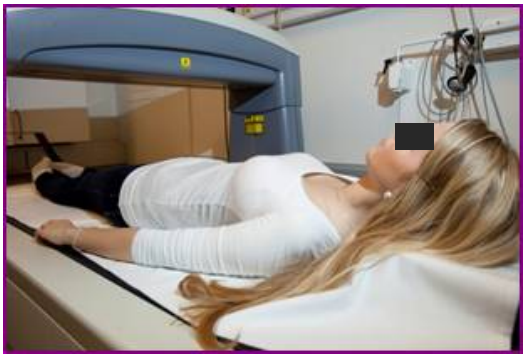

Figure 4 Demonstrating the dual energy X - ray absorptiometry from the Fit Futures study (32)

Laboratory Data

Non - fasting blood samples were collected and stored in the Biobank at the University Hospital of Northern Norway, at - 80 °C. By using high pressure liquid chromatography mass spectroscopy (LC-MS/MS), serum 25 - hydroxyvitamin D was analyzed, at the Hormone Laboratory, Haukeland University Hospital (26, 34). Subsamples were later re - analyzed using the LC-MS/MS method at the Cork Centre for Vitamin D and Nutrition Research, in order to standardize the results and make them comparable across different studies and countries. The standardization revealed even lower levels of 25 - hydroxyvitamin D than originally reported (26). The Cork Centre for Vitamin D and Nutrition Research, is certificated by the Centers for Disease Control and Preventions Vitamin D Standardization Certification Program (35) and monitored on an on - going basis by participation in the Vitamin D external Quality Assessment Scheme (36).

Combining MIH data with 25 - hydroxyvitamin D status

All participants with data available both from the MIH - study (3) as well as 25 - hydroxyvitamin D data and BMD measurements at total hip and femoral neck, were included. Data from blood samples and dual energy X - ray absorptiometry, regarding 25 - hydroxyvitamin D and BMD, respectively, were used to asses if there was any relation with the presence or absence of MIH. It must be emphasized that standardized values for 25 - hydroxyvitamin D were used in all analyzes.

Statistics

A logistic regression model was used to test if there was any statistical association between MIH affected, and not affected participants in BMD at hip and femoral neck, and levels of 25 - hydroxyvitamin D in separate analysis. MIH affected participants were in addition to this, divided into groups based on severity, but also groups based on numbers of affected teeth. These groups were tested the same way as the ordinary MIH affected participants in a logistic regression model compared with non - affected participants. Severe MIH affected participants were defined: FPM with PEB, atypical restorations and/or extractions. The analyses were performed sex stratified, and adjusted for age, height and weight, since BMD is influenced by these variables (32). The Statistical Package of Social Science software v. 26.0 (IBM SPSS Inc., Chicago, IL, USA) was used in all calculations and analyzes.

Results

The selection of participants

After selection of participants due to presence of relevant data for the present study, a total of 708 participants were included, illustrated in Figure 5. It was an outnumber of boys representing 54.1 % (n=383), and further 45.9 % (n=325) girls.

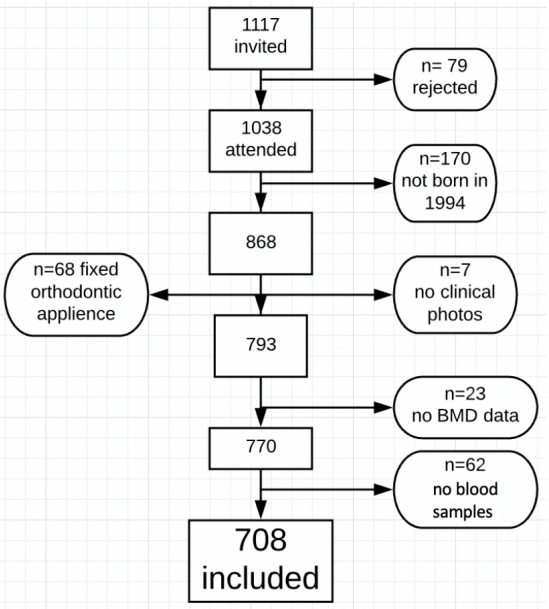

Figure 5: Flow chart for inclusion of participants

BMI measurements

BMI was in the range of 16.2 to 42.9 kg/m^2, with 22.3 kg/m^2 in total mean.

BMD measurements

When looking at mean BMD at total hip, in the range of 0.720 - 1.600 g/cm^2, boys had slightly higher values than girls. The same tendency were seen in mean BMD at femoral neck in the range of 0.730 - 1.600 g/cm^2, shown in table 1.

	Mean BMD total hip, g/cm^2	Mean BMD femoral neck, g/cm^2
Girls	1.069	1.077
Boys	1.118	1.107
Total	1.096	1.093

Table 1 Mean BMD at total hip and femoral neck in g/cm^2

25 - hydroxyvitamin D measurements

The levels of 25 - hydroxyvitamin D in the adolescents were widely distributed between 8.2 - 100.5 nmol/L. Both sexes had insufficient serum levels of 25 - hydroxyvitamin D, boys significantly lower on average than girls, 34.0 nmol/L and 43.7 nmol/L, respectively. A division of participants, sex stratified in regards to 25 - hydroxyvitamin D levels, is demonstrated in table 2.

Mean 25 – hydroxyvitamin D	> 50 nmol/L (sufficient)	< 50 nmol/L (insufficient)	Total
Girls	108	217	325
Boys	59	324	383
Total	167	541	708

Table 2 Number of participants (n) divided into groups, based on 25 – hydroxyvitamin D levels

The prevalence of MIH

A total of 98 participants were affected with MIH at varying degrees. Despite fewer girls than boys in the study, it was a higher number of girls with MIH (n=52), than boys (n=46). Thus, the prevalence of MIH was 16.0 % in girls, among boys 12.0 % and when counting for both sexes, 13.8 % .

Associations between BMD and MIH

When regards to BMD values at total hip and femoral neck, in separate analyzes, the MIH affected girls had lower values of BMD than not affected girls. The opposite was observed in boys, shown in table 3. No statistical associations was observed.

	No MIH affected participants (n=610)		MIH affected participants (n=98)		Participants with 3-4 affected FPM (n=23)		Participants with severely affected FPM (n=23)	
	BMD total hip mean, g/cm^2	BMD femoral neck mean, g/cm^2	BMD total hip mean, g/cm^2	BMD femoral neck mean, g/cm^2	BMD total hip mean, g/cm^2	BMD femoral neck mean, g/cm^2	BMD total hip mean, g/cm^2	BMD femoral neck mean, g/cm^2
Girls mean BMD	1.070	1.079	1.063	1.067	1.056	1.077	1.072	1.049
Boys mean BMD	1.117	1.106	1.132	1.113	1.118	1.124	1.063	1.027
Mean BMD (all)	1.096	1.094	1.095	1.089	1.081	1.095	1.068	1.039

Table 3 Mean BMD in no MIH affected participants, in affected participants, in participants with 3-4 MIH affected FPM and participants with severely affected FPM, sex stratified

Associations between 25 - hydroxyvitamin D and MIH

No significant difference was found between participants affected with MIH and not affected with MIH in regards to mean serum 25 - hydroxyvitamin D level, see table 4. The prevalence of MIH was higher in the group of participants with insufficient levels of 25 - hydroxyvitamin D (14.4 %) compared to the group with sufficient levels of 25 - hydroxyvitamin D (12.0 %), but no statistical difference was found here. In girls with 25 - hydroxyvitamin D < 50 nmol/L

the prevalence of MIH was 18.0 %, compared to 12.0 % when 25 - hydroxyvitamin D > 50 nmol/L. This might indicate that adolescent are more likely to be affected with MIH if having insufficient levels of 25 - hydroxyvitamin D, although the results is not statistically significant.

		Not MIH affected participants (n=610)	MIH affected participants (n=98)	Participants with 3-4 affected FPM (n=23)	Participants with severely affected FPM (n=23)
Mean 25 – hydroxyvitamin D in nmol/L	Girls	44.2	41.3	40.5	37.4
	Boys	33.9	34.6	32.2	32.0
	Total	38.5	38.2	37.2	35.1

Table 4 Mean 25 – hydroxyvitamin D in not MIH affected participants, in affected participants, in participants with 3-4 MIH affected FPMs and participants with severely affected FPMs, sex stratified

Number of MIH affected FPMs in regards to 25 - hydroxyvitamin D and BMD

The numbers of MIH affected FPM in each participants ranged from 1 - 4, categorized in table 5. Mean number of affected FPM in total was 1.9, in boys 1.7, and 2.0 in girls. Participants with 3 - 4 MIH affected FPMs, was split from individuals without any MIH – affected teeth, to relate if there was any difference in mean BMD and/or 25 - hydroxyvitamin D between the two groups. The levels of 25 - hydroxyvitamin D was decreased for both sexes with 3 - 4 MIH in comparison to the adolescents without MIH. The mean level for girls with MIH was 3.7 nmol/L less than in girls without MIH (table 4). No statistical association was found between BMD at total hip or femoral neck, neither for 25 - hydroxyvitamin D level and numbers of affected molars, even though corrected for BMI and sex.

Number of affected FPMs	0 (control group)	1	2	3	4
Girls	273	20	18	8	6
Boys	337	24	13	6	3
Total	610	44	31	14	9

Table 5 Numbers of affected FPMs

Severely MIH affected participants in regards to 25 - hydroxyvitamin D and BMD

MIH affected individuals can be categorized into different levels of severity. In this context, all participants with atypical restorations, PEB and those who had gone thru extractions caused by MIH on FPM, was described as severe. Of the 98 MIH affected participants, 13 girls and 10 boys were categorized as severe. Participants with opacities “only”, was on this basis excluded from the ‘severe selection’.

The severely affected participants did show lower 25 - hydroxyvitamin D levels, without any statistical relationships. Apart from mean BMD at total hip in girls, all mean BMD values (hip and femoral neck) were lower in severely affected participants compared to individuals without MIH, as displayed in table 3. An independent t - test did not show any statistical differences between participants affected by MIH and participants not affected by MIH, not even for severely affected MIH boys, in regards BMD levels at hip or femoral neck. However, in a binary logistic regression model, adjusted for sex, weight and height (BMI), boys severely affected by MIH had on average significantly ($P < 0.05$) lower BMD values at femoral neck compared to boys without MIH.

Discussion

In this study, we did not find any statistical significant differences in vitamin D levels or BMD between adolescents with and without MIH, although there was a tendency towards lower levels of both vitamin D and BMD in the affected groups. It is however worth noticing that there was a statistical difference in mean BMD levels at the femoral neck between boys severely affected by MIH and not MIH affected boys, after adjustments of height and weight. These adjustments are obligatory when analyzing BMD, since the scan area and skeletal size are influenced by these parameters, which explains the difference between the logistic regression model and the independent t – test results (37, 38). A study from 2016, analyzing BMDs and Bone Mineral Contents (BMCs) possible association with HSPMs and MIH affected participants at the age of six, did neither find associations between HSPMs nor MIH and BMD/BMC. When they adjusted for BA, they found a statistical association with lower BMC in participants with HSPMs, but not in participants with MIH (15).

Both femoral neck and total hip BMD was lower in boys severely affected with MIH compared to boys not MIH affected, however, statistical significant only at the femoral neck. We can only speculate in the reason why there was a difference between the measurement sites, but it is known that the total hip differs from the femoral neck by having a higher content of cortical bone (cortical bone has a higher density than trabecular bone). Also, the human body is 3 - dimensional, and therefore a 2 - dimensional scanning does not perfectly reflect the BMD, although the 2 - dimensional dual energy X - ray absorptiometry scan is the up to date gold standard for measuring BMD (39). Because of the anatomy, the BMD levels at the hip can be overestimated, explained by more overlaps of cortical bone at the hip using a 2 - dimensional scan area (37). These two measurement sites do therefore not necessarily represent the same, but since the direction of the findings were similar at the two sites, it may also reflect a power problem.

As in boys, the same tendency are seen in girls when regards to associations between MIH severity and mean BMD at femoral neck. In comparison there was no statistical difference in mean BMD at femoral neck between severely MIH affected girls and girls not affected with MIH. A greater proportion of girls would have been preferable, but it also could have been advantageous to have a larger selection of participants, to possible strengthen the results.

During growth there is alterations and increases in bone mass, and this gain of bone mass suggests to have a peak during puberty (40). At the time of BMD measurements, the adolescents was in the range of 15 - 17 year. It was not adjusted for puberty status in this study. The puberty factor can therefore be a cofounder, as girls in general are reaching puberty earlier than boys (41). Another article from the Tromsø study, tells that all girls had reached puberty at the time of BMD measurements, and most of the boys (32). Future studies should advantageously consider BMD measurements as early as reasonably achievable being more representative. Despite the age difference between the present study and *van der Tas* et al. (2016), we found similar tendencies (15).

Boys had on average, higher BMD values but lower prevalence of MIH, compared to girls in the present study. Boys is generally known to have higher BMD levels than girls. Several studies also conclude MIH being more prevalent among girls (3, 42). BMD may thus apply to appear to reflect the prevalence of MIH at group level in this study.

Very small differences in mean BMD values are seen in not MIH affected participants compared to the whole group of MIH affected participants independent of dual energy X - ray absorptiometry scan area. These results coincide with the results of from *van der Tas* et al. (2016), were they find negative correlation between BMD and MIH in 6 years old (15). BMD values are also reasonably unchanged between not MIH affected participants and the sample of MIH affected adolescents with 3 – 4 affected FPM in this study. The number of MIH affected teeth does not appear to play a significant role on BMD.

Although serum 25 – hydroxyvitamin D were lower in MIH participants, especially in severely affected individuals, no statistical association was found. This supports results from a cohort study, also by *van der Tas* et al. (2016), examining the same relationship in 6 years old (43). In another study an association is found where elevated 25 - hydroxyvitamin D level was associated with a lower prevalence of MIH (29). Interestingly, this was also recorded in this study, without a significant relationship.

It should be noted that "only" a small group 23/98 (22.5 %) of the participants with MIH was severely affected by MIH. This proportion of distribution is reasonable, seen in the context of an earlier study (44). This study is somewhat different from previous studies, being the first to split the participants into different groups based on MIH severity. *Kuhnish* et al. (2015) found

a negative correlation between elevated 25 - hydroxyvitamin D levels and MIH (29). The assumption for studying the most severely affected participants with MIH was to see whether the level of 25 - hydroxyvitamin D and BMD would correspond to the severity of MIH (29).

The weaknesses of this and earlier studies done within the same theme is that data of the content of 25 - hydroxyvitamin D in serum is missing during the period when affected teeth are mineralized. In order to find out the impact of 25 - hydroxyvitamin D on mineralization, the content of the vitamin during the period of mineralization period would have to be known. This period is limited and ongoing years before eruption of affected teeth. MIH is thereby not diagnosed until several years later. We therefore chose to use BMD as a marker of long - term exposure of 25 - hydroxyvitamin D (45). In this study it was therefore considered to look at BMD data from femoral neck and hip of the participants in order to see whether there were any connection between levels of BMD and presence of MIH.

It should be mentioned that BMD is only a proxy for 25 - hydroxyvitamin D status. BMD is influenced by a number of other factors such as genetics, nutrition and physical activity during childhood as well as conditions in fetal life (32, 46). These factors can therefore be cofounders in the BMD analyzes. The associations we observed between BMD and severe MIH affected participants in this study, may not necessarily be said to be due to 25 - hydroxyvitamin D. There might be other common factors that explain this association.

Participants were drawn from a whole grade level from 9 schools, with a high attendance rate. This assumes that the group of studied adolescents are highly representative. In order to have a harmonized group of participants, it was chosen to include only those born in 1994. This was done in order to make the selection of participants as similar as possible achievable. Although the number and selection of participant should be assumed to be a strength of this study, several participants was not included in the analyzes, due to lack of necessary data for our analyzes. Alternatively, to increase the number of selected participants for this present study, those missing blood test could have been included for BMD analyzes, and vice versa.

Conclusion

Mean BMD levels at femoral neck suggests to be lower in boys severely affected with MIH compared to boys with no MIH. A tendency can also be seen in mean BMD levels at hip for both sexes, although not significant. Even though the selection of participants are assumed to be representative, it would be preferable in future studies to have more BMD measurements - and blood sample - datas. Future studies could be recommended to divide the participants based on severity, and with this background investigate associations between BMD and MIH.

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