Effects of a 2-year early childhood vitamin D_3 intervention on tooth enamel and oral health at age 6-7 years

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Abstract

Introduction: The aim of the study was to compare the effects of a 30 µg/day vs 10 µg/day vitamin D supplementation, given during the two first years of life, on oral health at the age of six to seven years.

Methods: In 2013-2016, we conducted a randomized, double-blinded, clinical trial from age 2 weeks to 2 years of daily vitamin D_3 supplementation (10 vs 30 µg), including 975 healthy infants. For the present follow-up study at age 6-7 years, a sample of 123 children underwent oral examination by investigators blinded to the intervention group. Tooth enamel defect and caries findings, oral rinse active matrix metalloproteinase-8 levels, and tooth eruption were recorded. The intervention groups were compared with chi-square and Mann Whitney U tests. Associations of the oral health outcomes were evaluated with correlation analysis and logistic regression.

Results: Of the children (median age 7.4 years, 51% boys), 56% belonged to the 30 µg intervention group. Developmental defect of enamel (DDE) was found in 39% of the children in the 10 µg intervention group and in 53% of the 30 µg group ($p = 0.104$). In total, 94% of children were vitamin D sufficient (25(OH)D ≥50 nmol/l) and 88% had caries-free teeth. No associations were found between vitamin D intervention group in infancy and oral health or the presence of DDE.

Conclusion: Daily supplementation with 10 µg vitamin D_3 in the Northern Hemisphere seems adequate in healthy children younger than 2 years in ensuring good oral health at early school age.

Trial registration: ClinicalTrials.gov (NCT04302987)

Introduction

Tooth enamel and dentin, unlike bones, do not remodel [1]. Therefore, developmental defects of enamel (DDE), independent of the causative factor, relate to a time-window of a particular tooth crown formation, ranging from intrauterine period to 11 postnatal months for primary teeth. Regarding earliest permanent teeth, the first permanent molars mineralize between birth and 3 years, and incisors between 3 months and 5 years [2]. Hypocalcemia secondary to vitamin D deficiency is one of numerous causes of DDE [3–7]. Decreased prevalence of DDE but no change in caries status was reported in offspring of mothers on high-dose vitamin D_3 supplementation (70 µg/day;2800 IU vs 10 µg/day;400 IU) during pregnancy [8].

To explore whether high-dose vitamin D supplementation associates with oral and dental health also when administered postnatally, we performed an oral health examination at age 6-7 years among participants of a randomized, double-blinded, 24-month clinical trial of daily vitamin D_3 supplementation of 10 or 30 µg from 2 weeks to 2 years of age, recruited in 2013-2014 [9]. The examination included a strip test for active matrix metalloproteinase (MMP)-8. MMP-8 not only positively correlates with dental caries and the degree of inflammation in periodontal disease [10–13], but also with cord blood 25(OH)D in healthy newborns, reflecting immunomodulatory functions of vitamin D in the innate immune response [14]. We hypothesized that 1) high-dose vitamin D supplementation in infancy associates with decreased occurrence of DDE but does not affect caries prevalence, and 2) active MMP-8 in oral rinse positively correlates with serum 25(OH)D concentration, ongoing tooth transition, and caries lesions extending to dentin.

Materials and Methods

Sample characteristics

The current study was a follow-up sub-study of the Vitamin D Intervention in Infants (VIDI) study that was a randomized, double-blinded, 24-month clinical trial of daily vitamin D_3 supplementation of 10 or 30 µg administered to healthy infants from 2 weeks to 2 years of age, between the years 2013-2016 [9,15]. The original study group consisted of 975 infants, born to mothers of Northern European origin in Helsinki, Finland. Recruitment procedures, as well as inclusion and exclusion criteria of VIDI study have been reported previously [9]. At birth, 96% of the children were vitamin D sufficient (25[OH]D concentration ≥20.03 ng/ml) [15]. All the children who had participated in a follow-up visit at age 2-years were invited to the present follow-up study, and the final study cohort consisted of those willing to participate. The children, aged six to seven years, were invited to a clinical and laboratory examination between 2019 and 2021. Only children with complete baseline data on the vitamin D supplementation were included in this study. Two enrolled children with missing baseline data were excluded from the study. The VIDI trial and the follow-up study were approved by Research Ethics Committee of the Hospital District of Helsinki and Uusimaa (107/13/03/03/2012, HUS/1541/2019), and written informed consent was obtained from the participants' parent/legal guardian/next of kin to participate in the study. The current study was approved by the Children's Hospital, Helsinki. The trial is registered in ClinicalTrials.gov (NCT04302987). STROBE guidelines are followed.

The current study is a secondary analysis of the VIDI follow-up study. Here, the primary outcome was molar-incisor hypomineralization (MIH), the most prevalent type of DDE [16,17]. As reference for sample size calculation, we used a reported prevalence rate of 21% for MIH in the urban Finnish areas [18]. The calculation was based on the ability to detect a clinically relevant difference in the occurrence of MIH between the two trial arms (3% vs 21% with α= 0.05 and power of 80%). Sample

size calculation indicated that 50 participants were required in both subgroups. Total sample size was increased to 123 to ensure enough participants in each subgroup and account for losses in eligibility. Sample numbers were expected to be unequal between the subgroups as the researchers were blinded to previous intervention.

Reliability of oral evaluation

We applied the WHO Basic Methods for Oral Health Surveys -standardization guidelines to increase reliability and repeatability of the observations [19]. WHO guidelines recommend that the calibration should include at least 25 independently examined subjects and the consistency level reach 85% [20]. In our study, the primary investigator was evaluated against gold standard of an experienced pediatric specialist dentist (benchmark scorer) to evaluate interrater reliability of dental hard tissue examination. Both investigators independently examined 26 children having altogether 585 teeth. Calibration was undertaken for the following clinical variables; presence/absence of DDE, MIH, plaque, and caries lesions, as well as classification of the size of hypomineralization lesion. Validation data was a random sample of the main study group. To avoid loss of efficiency outside the validation data set, as suggested by Agbaje et al., all further enamel defect findings were either clinically examined independently by both investigators (49 subjects) or examined from clinical photographs (25 subjects) to verify diagnosis of DDE or MIH [21].

Oral examination

Clinical examination protocol and criteria were formulated in line with previous studies [22–24]. An oral health risk assessment tool -questionnaire was completed by all but one of the child's parent/caretaker. The questionnaire followed the protocol of the American Academy of Pediatrics with a modified translation of Oral Health Questionnaire as applied by Rajavaara and colleagues [25].

Additionally, parent/caretaker was asked whether the child had been treated with antibiotics during the first two years of life, whether the child uses xylitol products, and/or vitamin D supplementation.

A full dental status was compiled by one experienced dentist. Eruption status of the first permanent molars and permanent incisors was graded as un-erupted, partially erupted, or fully erupted. Missing teeth and fixed orthodontic appliances were recorded. Each tooth was examined for enamel hypomineralization or hypoplasia, caries lesions extending to dentin, fillings, and atypical restorations. Recording was based on a two-step system: first a tooth-level code was provided and then a surface-level code. Five surfaces (occlusal, facial, lingual, mesial, and distal) were assessed for each tooth.

Visible plaque on the facial surface of a front tooth in children indicates a caries risk [26]. Thus, dental plaque was visually assessed on maxillary right first incisor, primary or permanent, as present or not present. Dental caries status was defined as decayed and/or filled teeth. The sum of the number of decayed, missing due to caries, and filled primary or permanent teeth (dmft/DMFT score), was calculated for each patient. In principle, the score value ranges from 0 to 24 in a full early mixed dentition. Non-carious enamel defects that were of 1 mm or more in diameter, were recorded and defined according to the European Academy of Paediatric Dentistry criteria (type and extent codes listed in Supplementary Table online) [16]. Children with at least one permanent first molar affected by characteristic hypomineralization were considered to have MIH. DDE was diagnosed when enamel hypomineralization was limited to other primary or permanent teeth or appeared diffuse. If more than one condition was present (e.g. diffuse opacity and hypoplasia), the extent and type of the largest one was recorded.

Active MMP-8 in oral fluid was tested with a non-invasive chairside point of care (POC) lateral flow immunoassay test (PerioSafe®, Dentognostics GmbH, Jena, Germany) in accordance with the manufacturer's instructions, visually read either as positive (aMMP-8 > 20 ng/ml) or negative result.

Laboratory analyses of biochemical markers

Serum 25(OH)D concentration was measured of 114 (93%) and parathyroid hormone (PTH) concentration of 108 participants (89%). Serum 25(OH)D and PTH concentrations were analyzed with automated multi-discipline immunoassay system with chemiluminescence detection (IDS-iSYS™, Immunodiagnostic Systems Ltd., Bolton, UKIDS Ltd., Bolton, UK) at Pharmatest Services Ltd. (Turku, Finland). Previously obtained cord blood MMP-8 concentrations [14] as well as ionized calcium and phosphate concentrations in venous blood at 12 months [27] were compared against the current findings.

Statistical methods

Kolmogorov-Smirnov test for normality was applied to assess the distribution of the data. Chi-square (χ^2) test was used to compare the enamel defect findings and MMP-8 activity between the two intervention groups. Mann Whitney U test was applied to compare the dmft/DMFT score between the intervention groups. Point-biserial correlation was computed to assess the relationship between the presence of DDE/MIH, dmft/DMFT score, atypical dental restorations, aMMP-8, calcium and phosphate concentrations, presence/absence of plaque, number of daily meals and tooth brushing, usual drink with meals and for thirst, xylitol use, use of fluoride toothpaste, serum 25(OH)D, and PTH concentration. Serum 25(OH)D, PTH, calcium, and phosphate were analyzed as continuous variables. A logistic regression was performed to ascertain the effects of sex, use of antibiotics, and vitamin D intervention on the likelihood that participants have DDE or MIH. A p-value less than 0.05 was

deemed statistically significant. Statistical analyses were performed with IBM SPSS Statistics software (version 27).

Results

The final sample consisted of 123 children aged between 6.5 and 7.9 years (median 7.4 years) at the time of the examination (Fig. 1). Of the participants, 63 (51%) were boys and 60 girls (49%). Regarding the earlier vitamin D intervention, vitamin D supplement of 10µg/day had been given to 54 (44%) children and a 30µg/day dose to 69 (56%) children until two-years of age. Characteristics of study participants as well as oral examination findings and biochemical parameters are presented in Table 1, and data on oral health questionnaire in Table 2. According to the recollection of the parents, 46% of the children in the 10 µg/day vitamin D_3 intervention group and 62% of the children in the 30 µg/day vitamin D_3 intervention group had received antibiotics during the first three years of life. Vitamin D supplementation was used by 96% of the children in the 10 µg/day vitamin D_3 intervention group and 93% of the children in the 30 µg/day vitamin D_3 intervention group (Table 2). Parents reported an average age of 7 months for eruption of the first deciduous tooth (range 3 to 12 months) for children in both intervention groups. None of the children had orthodontic treatment at the time of the examination.

Interrater agreement of dental hard tissue examination was good (95%). DDE was found in dentition of 39% of the participants in the 10 µg/day vitamin D_3 intervention group and in 53% of the participants in the 30 µg/day intervention group (Table 1). MIH was found in dentition of 13% of the children in the 10 µg/day vitamin D_3 intervention group and in 14% of the participants in the 30 µg/day intervention group (Table 1). Largest MIH lesions covered more than 2/3 of the tooth surface in 2 subjects of the 10 µg/day vitamin D_3 intervention group and in 3 of the 30 µg/day intervention

group (photoimage in the Supplementary Table Online). No association was found between vitamin D intervention group and presence of DDE ($\chi^2(1)= 2.639$, $p = 0.104$) or MIH ($\chi^2(1)= 0.06$, $p = 0.807$). Similarly, sex, vitamin D intervention group, or use of antibiotics were not associated with DDE or MIH in the logistic regression model. In total, the dmft/DMFT score was zero in 88% of the participants indicating good oral health. However, visible plaque and debris accumulation on the facial surface of an upper incisor tooth was detected in 20% of the participants. Three children, one in the 10 µg/day intervention group and two in the 30 µg/day intervention group, displayed active caries lesions extending to dentin. Atypical restoration of a deciduous molar was present in one child of the 30 µg/day vitamin D_3 intervention group. The dmft/DMFT score was not different between the intervention groups (n=123, U=1902, p=0.726). Mouthrinse aMMP-8 POC test was positive in 30% of the participants; 28% of those in 10 µg/day vitamin D_3 intervention group and 32% in 30 µg/day vitamin D_3 intervention group. Thus, no association was found between vitamin D intervention group and a positive aMMP-8 result ($\chi^2(1)= 0.243$, $p = 0.622$).

At the time of dental examination, 94% of the children were vitamin D sufficient (25(OH)D ≥50 nmol/l, n=107/114) (Table 1). Only seven subjects (6%) displayed insufficient vitamin D status (25(OH)D<50 nmol/l); four in the 10 µg/day intervention group and three in the 30 µg/day vitamin D_3 intervention group. Serum PTH concentration was within the laboratory reference range of 15-65 pg/mL in 104 subjects of 108 (96%) with available data. No correlations existed between DDE/MIH and 25(OH)D or PTH concentration (p>0.05). Similarly, presence of DDE/MIH did not correlate with dmft/DMFT score, aMMP-8-test result, presence/absence of plaque. No correlation existed between DDE/MIH or dmft/DMFT score and any of the self-evaluated oral heath parameters (p>0.05) (Table 2). The previously documented serum calcium and phosphate concentrations at 12 months did not correlate with presence of DDE in permanent teeth ($r_{pb(119)}$=-0.1570, p=0.088 and $r_{pb(96)}$=-0.009, p=0.928).

Of the subjects, 67 (54%) presented with erupting teeth. Number of erupting teeth correlated positively with present serum 25(OH)D concentration ($r_{pb(96)}$=0.210, p=0.039), but not with PTH (p>0.05). Total number of erupting and erupted permanent teeth did not correlate with serum 25(OH)D concentration. The number of deciduous teeth did not correlate with vitamin D intervention group or present serum 25(OH)D concentration. Clinically observed plaque showed no association with the aMMP-8 level in mouthrinse (p>0.05). Neither correlated the aMMP-8 activity with the number of erupting teeth or daily use of xylitol products. However, a small negative correlation existed between aMMP-8 and use of xylitol ($r_{pb(120)}$= -0.199, p=0.028). The previously documented cord blood MMP-8 concentration did not correlate with aMMP-8 in oral rinse ($r_{pb(122)}$= 0.117, p=0.199).

Discussion

Our study contributes to the Developmental Origins of Health and Disease hypothesis [28], by discovering that vitamin D supplementation dose during infancy might not be among the environmental factors affecting tooth enamel development. In 123 healthy children, with overall good oral health, there was no association between 2-year vitamin D intervention dose in infancy and the presence of developmental defects of enamel (DDE) or oral health at the age of six to seven years. The prevalence of DDE was 47% in the whole study cohort and 54% in the children with a daily vitamin D_3 supplementation of 30 µg until age two years. In previous studies, maternal intake of vitamin D during pregnancy associated with lower occurrence of autoimmune disorders and allergic outcomes in offspring by age five, suggesting a long-lasting immunomodulatory role of vitamin D [29,30].

The mean prevalence of molar-incisor hypomineralization (MIH) in whole of Finland is 18% and in urban areas 21% [18,22]. As the etiology of MIH is by large unknown, prevention is currently impossible [8,31]. The condition varies in severity and can cause hypersensitivity of the affected teeth, post-eruptive breakdown of the porous enamel leading to acute symptoms as well as rapid carious degradation, and a need of restorative treatment or even tooth extraction [17]. In the current cohort, interestingly, the prevalence was lower (14%) than in previous studies for MIH in an urban area [18,32]. This likely relates to causative factors, other than vitamin D_3 status, in the development of MIH.

While it is widely recognized that enamel defects are one manifestation of the biochemical abnormalities associated with rickets, the previously described findings concerning the association between vitamin D status, MIH, and caries are somewhat contradictory [3,6,33,34]. A previous study proposed that vitamin D deficiency-related hypocalcemia during enamel formation correlates with enamel hypoplasia in primary dentition [6]. Furthermore, vitamin D supplementation of 60 µg/day during pregnancy has been suggested to reduce the prevalence of enamel defects in the offspring, with an approximately 50% reduced odds, while not affecting caries occurrence at age 6 years [8]. In contrast, in an uncontrolled study by Kühnisch et al., vitamin D supplementation (dose not reported), during the first year of life was not associated with occurrence of MIH, but reduced caries in primary dentition [35]. Similarly, no association was found between umbilical cord blood 25(OH)D concentration and the presence of MIH at the age of six in a study by van der Tas et al. [33]. In the current cohort, neither the supplemental dose of vitamin D during the first two years of life or serum 25(OH)D concentration at age 6 to 7 years correlated with MIH or DDE. The latter finding is expected, as mineralization has completed during the first years of life [36]. Our cohort comprises healthy term-born children with sufficient vitamin D status from birth, which may at least partly explain why we did not find any effect of early-life vitamin D intervention, or 12-month calcium and phosphate

levels on enamel defects, the etiology of which remains obscure. Daily vitamin D supplementation of 7.5 µg is recommended to every child aged 2 to 17 years in Finland. In total, 94% of the parents of children in our cohort, reported that the child used vitamin D supplementation.

The role of antibiotics in etiology of MIH is controversial, but the use of certain antibiotics, particularly amoxicillin, has been associated with elevated risk of MIH [37–39]. Our study found no association between antibiotic use and MIH. In contrast to earlier findings [22,40], we were not able to observe an association between MIH and caries but notably in our study, the children were young, with very recently erupted first permanent molars.

Normal tooth eruption includes axial movement of a tooth in alveolar bone, a process that requires the action of PTH. PTH, together with vitamin D, regulates mineral metabolism, and influences bone remodeling and periodontal tissues [41,42]. Vitamin D deficiency is a risk factor for delayed tooth eruption associated with persisting primary tooth [43]. We found a weak but statistically significant positive correlation between serum 25(OH)D concentration at age 6-7 and the number of erupting teeth, but not with the counts of either primary or fully erupted permanent teeth as such, or in combination with the number of erupting teeth. Hence, there seems to be a correlation between the 25(OH)D concentration and ongoing tooth eruption, but no evidence of either accelerated or delayed dental development in this group of healthy children.

Matrix metalloproteinase (MMP) endopeptidases regulate cell matrix composition, participate in morphogenesis, tissue remodeling, and immune response owing to their anti-inflammatory capacity [44]. In adults, mouthrinse POC aMMP-8 test, based on one of the genetically distinct but structurally

similar proteinases present in the family of 23 MMPs, is feasible in determining the severity and course of active periodontal disease [10,45] and detecting emerging and existing diabetes [46]. Moreover, studies implicate roles for MMP-8, also known as collagenase-2, in remodeling of the fibrous tooth-supporting tissue, periodontium, during tooth eruption [47,48] and orthodontic tooth movement [49], as well as in degradation of the collagenous dentin matrix with progression of caries [12]. Hence, due to the presence of these potential confounding factors, testing for aMMP-8 has not been considered sensitive for periodontal-disease risk in children. Progression of caries in early childhood is rapid [50]. Vitamin $D_{3,}$ as an inhibitor of MMP-8, could have slowed down the progression of early childhood caries during the first two years of life. That would be reflected as a less decayed dentition years after, at the age of 6-7, and consequently, as lower oral fluid aMMP-8 levels. Nonetheless, in our cohort, no statistically significant association was detected between aMMP-8 level and the number of erupting teeth or active caries lesions. Notably, however, 88% of the study subjects displayed caries-free teeth. A previous study, on a random sample of Finnish 6-year-old children, found a lower 68% prevalence of caries-free children [51]. None of our study subjects had fixed orthodontic appliances. Neither presence of plaque nor any of the indicators of oral health behavior and general health habits, or earlier cord blood aMMP-8 concentration or vitamin D supplementation dose, showed an association with oral fluid aMMP-8. Interestingly, in our study, subjects using xylitol (birch sugar) products displayed a positive aMMP-8 test result less often than those not using xylitol.

Development of oral microbiome begins after birth [52,53]. Erupting dentition is a determinant of distinct age-related bacterial colonization in children [52]. In all age groups, gingival inflammation, gingivitis, is very common [54,55] and occurs as a response to bacteria [56]. Periodontitis, leading to destruction of the periodontal ligamentous and bony tooth-supporting structures, on the other hand, is rare in healthy children, but encountered in disorders such as Down syndrome [53,57]. PerioSafe®

chair-side oral rinse immunotest for aMMP-8 has been successfully validated to differentiate between gingivitis and periodontitis [58], and periodontal health and disease in adolescents and adults with permanent dentition [10,46,59]. However, to our knowledge, no prior documents have been published on its use in children with mixed dentition. Our findings suggest that aMMP-8 POC test seemingly is applicable also in children as the results showed no association with the tooth eruption status. This finding preliminarily implies that rapid and non-invasive oral rinse POC technology could be used in screening of children with underlying systemic conditions, and at risk for undiagnosed early emerging periodontitis, to refer them to a dentist for examination. Prospective studies are needed to verify the applicability of aMMP-8 POC test in diagnosing pre-periodontitis in children.

This study has some limitations. Families, with an interest on ensuring their child's health and wellbeing would be more likely to participate in follow-up studies. A larger sample size would more closely approximate the population and increase precision and reliability of the results. As a follow-up of a randomized clinical study, the strength of our study is the ability to determine treatment-predictive variable interaction.

In line with previous findings from VIDI study that did not show significant benefits for high-dose vitamin-D supplementation during the two first years of life [60], the present study found no benefit in forms of improvement in child's oral health or decrease in the occurrence of developmental defects of enamel in permanent incisors and first molars. In conclusion, daily supplementation with 10 µg vitamin D_3 seems adequate in children younger than 2 years also from an oral health perspective.

Statements

Statement of Ethics

This research complies with internationally-accepted standards for research practice and reporting. The VIDI trial and the follow-up study were approved by Research Ethics Committee of the Hospital District of Helsinki and Uusimaa (107/13/03/03/2012, HUS/1541/2019), and written informed consent was obtained from the participants' parent/legal guardian/next of kin to participate in the study. The current study was approved by the Children's Hospital, Helsinki. The trial is registered in ClinicalTrials.gov (NCT04302987).

Conflict of Interest Statement

The authors have no conflicts of interest to declare. The sponsors had no involvement in study design, the collection, analysis, and interpretation of data, the writing of the report; and/or the decision to submit the manuscript for publication.

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Author Contributions

Conceptualization and study design (Heidi Arponen, Janna Waltimo-Sirén, Sture Andersson, Outi Mäkitie, Elisa Holmlund-Suila), Methodology (Heidi Arponen, Janna Waltimo-Sirén, Timo Sorsa, Taina Tervahartiala, Outi Mäkitie, Elisa Holmlund-Suila), Investigation (Heidi Arponen, Janna Waltimo-Sirén, Helena H Hauta-alus, Mikaela Tuhkiainen, Taina Tervahartiala), Supervision (Janna Waltimo-Sirén, Timo Sorsa, Sture Andersson, Outi Mäkitie, Elisa Holmlund-Suila), Funding acquisition and resources (Heidi Arponen, Helena H Hauta-alus, Timo Sorsa, Sture Andersson, Outi Mäkitie, Elisa Holmlund-Suila), Data analysis (Heidi Arponen, Janna Waltimo-Sirén, Mikaela Tuhkiainen, Timo Sorsa, Taina

Tervahartiala, Sture Andersson, Outi Mäkitie, Elisa Holmlund-Suila), Writing (Heidi Arponen, Janna Waltimo-Sirén, Helena H Hauta-alus, Timo Sorsa, Taina Tervahartiala, Sture Andersson, Outi Mäkitie, Elisa Holmlund-Suila). All authors have approved the manuscript.

Data Availability Statement

Data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author. Cord blood parameters of the study subjects have been previously documented in Rosendahl et al. 2017 and Valkama et al. 2017 as referenced.

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Figure Legend

Fig. 1. Sampling of study subjects. Enrollment of the VIDI study subjects, and exclusion criteria are described in detail in Rosendahl et al. (JAMA Pediatr 127:646-54, 2018).

Table 1. Characteristics of the 123 participants at 6 to 7 years of age and oral health parameters by vitamin D supplementation during the two first years of life.

	Vitamin D supplementation 10 µg/day N=54				Vitamin D supplementation 30 µg/day N=69				Difference between groups
	N	percent	mean	SD	N	percent	mean	SD	P value
Sex (boy/girl)	25/29				38/31				0.334[#]
Age at examination (year)			7.4	0.2			7.4	0.2	0.846[##]
Number of fully erupted permanent teeth			9	3			9	3	0.733[##]
Number of erupting teeth			1	1			1	1	0.451[##]
DDE observed in the child	21	39%			37	53%			0.104[#]
In affected children, DDE % of teeth			13	13			10	5	0.763[##]
MIH observed in the child	7	13%			10	14%			0.807[#]
In affected children, MIH % of teeth			19	14			17	11	0.740[##]
MIH categorized by largest lesion size or atypical restoration I II III Atypical restoration	 2 2 2 0	 4% 4% 4% 0			 6 1 3 1	 9% 1% 4% 1%			
dmft/DMFT=0* dmft/DMFT=1 dmft/DMFT=2 dmft/DMFT>2	48 6 0 0	89% 11% 0 0	0	0	60 8 1 0	87% 12% 1% 0	0	0	0.726[##]
Visible plaque on permanent right incisor, upper or lower	9	17%			15	22%			0.481[#]
Active caries lesions	1	2%			1	1%			0.861[#]
Positive PerioSafe® test	15	28%			22	32%			0.622[#]

result (active MMP-8 biomarker)									
Serum 25-hydroxyvitamin D concentration (nmol/L)	53		72	17	61		74	15	0.532##
Serum parathormone (PTH) concentration (pg/mL)**	51		35	12	58		32	11	0.171##

DDE Developmental defects of enamel
MIH Molar incisor hypomineralization
*dmft/DMFT Sum of decayed, missing and filled primary and permanent teeth. Index score minimum value is 0 and maximum 24 in this age group with a maximum of 24 teeth in an early dentition
PerioSafe® Tests for active MMP-8 biomarker (Chi-square test)
** laboratory reference range 15-65 pg/mL
# Chi-square test
Mann-Whitney U test

Table 2. Results of the oral health questionnaire and self-reported history of antibiotics use.

	Vitamin D supplementation up to age of 2 years		
	N of respondents 10 vs 30 µg/day	10 µg/day (%)	30 µg/day(%)
Dental treatment in general anesthesia	54/68	1	0
Nursing bottle none, exclusively breastfed bottle up to 12 months bottle up to 24 months bottle over 24 months	54/69	 43 46 11 0	 54 43 3 0
Nursing bottle at nights up to 24 months	51/65	39	25
Contents of nursing bottle at nights milk water cocoa juice other	20/16	 100 6 0 0 0	 81 38 0 6 0
Occasional use of xylitol products	54/67	98	99
Daily use of xylitol	54/65	74	78
Use of vitamin D supplementation	54/69	96	93
Daily use of vitamin D supplementation	53/64	57	67
Number of meals per day three four	54/67	 7 93	 3 97
Number of snacks per day one two three	53/67	 83 17 0	 82 16 1
Usual drink for thirst water milk juice other	53/68	 96 9 2 0	 94 16 3 0
Frequency of daily tooth-brushing less than once once twice	53/68	 0 13 87	 0 9 91
Child brushes teeth him/herself (yes)	52/65	50	52

Use of electric toothbrush (yes)	53/67	87	90
Use of fluoride toothpaste (yes)	53/67	98	100
Antibiotics use before age 5 (yes)	41/59	61	73

N= 54 in the 10 µg/day vitamin D supplementation group
N=69 in the 30 µg/day vitamin D supplementation group