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ORIGINAL ARTICLE

The influence of infantile thiamine deficiency on primary dentition

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Abstract
Objectives The present study explored the histological and chemical effects of infantile thiamine deficiency (ITD) on enamel development through the examination of exfoliated deciduous teeth from a patient who had been fed during his first year of life with a thiamine-deficient milk substitute.
Materials and methods Ground sections derived from six exfoliated primary teeth were examined. Slices from a light microscope were photographed for histological analysis. We calculated the time when the amelogenesis insults occurred, and the data were cross-examined with the patient's medical history. We then measured the enamel content of calcium, phosphate, oxygen, carbon, and magnesium on two lines from the dentino-enamel junction (DEJ) to the outer surface using an energy dispersive X-ray spectrometer.
Results Carbon (organic matter) concentration in postnatal enamel was 2.37 times higher in ITD, phosphate levels were lower, and magnesium and calcium levels tended to be higher in ITD teeth.
Conclusion Chemical and histological analysis enabled us to confirm that thiamine deficiency in infancy impaired postnatal amelogenesis and resulted in less calcified enamel with a higher level of organic matter. Higher postnatal enamel carbon and magnesium concentration found in ITD may derive from either impaired mineralization caused by disturbed cellular metabolism or indirect damage to the ameloblasts due to the physical condition. Ca/P mean ratio in ITD teeth was higher than the mean ratio in the control displaying a damaged mineralization process.
Clinical relevance This is probably the first description of infantile thiamine deficiency effect on amelogenesis resulting in less calcified enamel.

Keywords Infantile thiamine deficiency · Striae of Retzius · Ameloblasts · Beriberi

Introduction

Infantile thiamine deficiency (ITD) occurs mainly in breastfed infants of mothers with inadequate thiamine intake and is hardly seen in formula fed infants with no underlying disease or malnutrition state [1–3]. An essential vitamin for brain development in infants [4, 5], thiamine deficiency usually develops within 12 weeks of a deficient intake [1] and includes nervous system (dry beriberi, or Wernicke-Korsakoff syndrome) or cardiovascular manifestations (wet beriberi) that may be lethal (Shoshin beriberi, Wernicke's encephalopathy) [6]. The best characterized form of thiamine (i.e., vitamin B_1) is thiamine pyrophosphate (TPP), a coenzyme in the catabolism of sugars and amino acids. Thiamine is required for the biosynthesis of the neurotransmitter acetylcholine and gamma-aminobutyric acid (GABA), and TPP is essential for activating the pyruvate dehydrogenase complex, an important component of the citric acid cycle and nucleotide synthesis [7].

Between October and November 2003, 20 infants with encephalopathy were hospitalized in pediatric intensive care units in Israel with various cardiac and neurological symptoms and two of them deceased [8]. Clinical signs consisted of constipation, agitation, apathy, vomiting, and lack of appetite

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followed by diarrhea, grunting, nystagmus, convulsions, and unconsciousness. All infants have been fed with a non-dairy soy-based infant formula (Remedia Super Soy 1 baby formula). Product analysis indicated that thiamine was "undetectable," rendering the formula thiamine deficient. The Israeli ministry of health identified 156 more infants who were fed the specific soy-based formula, eight of them had a mild degree of thiamine deficiency (TPPE <15 %) although all were receiving solid food products that contained thiamine in addition to the formula. Most thiamine deficiency-related symptoms disappeared completely within 2 to 9 weeks of treatment with thiamine [6]. Normal development was documented in all of these patients, with no signs of neurologic involvement. Five years follow-up showed that the children who suffered from thiamine deficiency presented psychomotor retardation, hearing loss, ataxia, stuttering, and limb pain [8]. Those symptoms appeared even when they had been fed with the defected formula for only a short time and had minor symptoms during infancy. Recent research [9, 10] found that 80 % of "Remedia infants" suffered from linguistic impairment, and 50 % had a motor developmental disorder, without intelligence deficits.

The effect of thiamine deficiency on the developing primary dentition has not been studied.

Enamel mineralization of the primary dentition begins during the 4th month in utero and ends during the first year of life [11]. Changes in ameloblasts' environment impair their function and result in enamel structural defects that affect the quantity and the integrity of the organic matrix [12], the orientation and growth of the apatite crystals, and the degradation and removal of the organic matrix [13, 14]. When ameloblasts are subjected to a destructive episode of either internal or external origin, a temporary change in the rhythmic enamel matrix formation (secretion or mineralization) may occur, causing some striae of Retzius (i.e., distinctive bands of malformed enamel that can be seen histologically) to be salient [15]. One such accentuated Retzius line corresponds to the event of birth and is known as the neonatal line [16] that marks the transition from intra-uterine to postnatal development [17]; others are known as calico-traumatic lines.

The present study analyzed the histological and chemical effects of ITD on enamel development through the examination of exfoliated deciduous teeth from a patient who suffered from ITD.

Materials and methods

Exfoliated deciduous teeth from a patient (AM) who suffered from ITD were examined. The medical history of the patient is summarized in Table 1.The patient had arrived to the Department of Pediatric Dentistry, the Hebrew University—Hadassah School of Dental Medicine, Jerusalem, Israel when he was 9.5 years old with cavitated mandibular first molars.

Table 1 Medical history of AM

0–4 m 2w	Breast fed.
>4 m 2w	Fed with soy-based infant formula.
5 m 3w	Daily vomiting.
6 m	Hospitalized for 17 days. Diagnosed as allergy to soy.
6 m 3w	Fed again with soy-based infant formula at home, together with solid food
7 m 2w	Diagnosed as ITD. B1 shots for 10 days. Change of formula. Examined by an orthopedist, cardiologist, ophthalmologist, and a pulmonologist. He suffered from irritability, lethargy, developmental delay, and motor retardation. He did not get any other medical treatment besides physical therapy and neurologist and psychologist surveillance.
8 m 1w	A single episode of vomiting. No treatment.
14 m	Hospitalized for 2 days after 3 weeks of apatite loss. No findings.

He was treated and is still followed up. The residual damage known comprises of motor difficulties and attention deficit disorder (ADD).

Study material

This study was carried out on ground sections derived from six exfoliated primary teeth of the patient: 53, 55, 63, 73, 75, and 84, and six match-paired teeth from healthy children (the control group) that were extracted during routine dental treatment for orthodontic reason.

Teeth preparation

All analyses were carried out on ground sections prepared from the teeth, using a Beuhler isomet diamond wafering blade saw. The teeth were embedded in acrylate according to the protocol of Caropreso et al. [18], and two 200 μm thick bucco-lingual sections were obtained from each tooth using a standard procedure in which in the molars one section was made through the tips of the mesial cusps and one through the tips of the distal cusps, while in the canines one section was made through the long axis. In the molars, accuracy of the plane of each section was inspected using a low-power binocular microscope to determine if the section traversed both the cusp tips. The sections were then polished using a 600 grit polishing paper to a thickness of 200 μm.

Histological analysis

Slices were photographed from a light microscope using ×20 enlargement. We identified the neonatal line in the enamel of all ITD and normal teeth. All ITD teeth demonstrated several

calico-traumatic lines in the postnatal enamel, with the second primary molar showing the highest number (Fig. 1). On this tooth, we measured on the dentino-enamel junction (DEJ) line the length from the point where the neonatal line meets the DEJ to the cemento-enamel junction (CEJ) point. This length indicates 12 months of growth. We then measured the distance on the DEJ line between the point of the neonatal line to the points where the calico-traumatic lines meet the DEJ and calculated the time when the amelogenesis insult occurred. The data was cross-examined with the patient's medical history.

Chemical analysis

The main elements in the enamel were identified by using scanning electron microscopy (SEM; Quanta 200, OR, USA) in a low vacuum mode in conjugation with an energy dispersive X-ray spectrometer (EDS). We measured the chemical content on two lines from the DEJ to the outer surface: the incisal and the cervical lines. The incisal line was located 0.5 mm apically from the dentin cusp, and the cervical line was located 1 mm occlusal to the CEJ (Fig. 2). The lines were perpendicular to the DEJ line. On each line, 300 points were measured from the DEJ outward. On each line, the location of the neonatal line was determined, and the 300 points were divided into prenatal and postnatal enamel chemical content. The results were recorded in Mol. wt% (molecular weight) units. The main elements for the analysis were calcium, phosphate, oxygen, carbon, and magnesium.

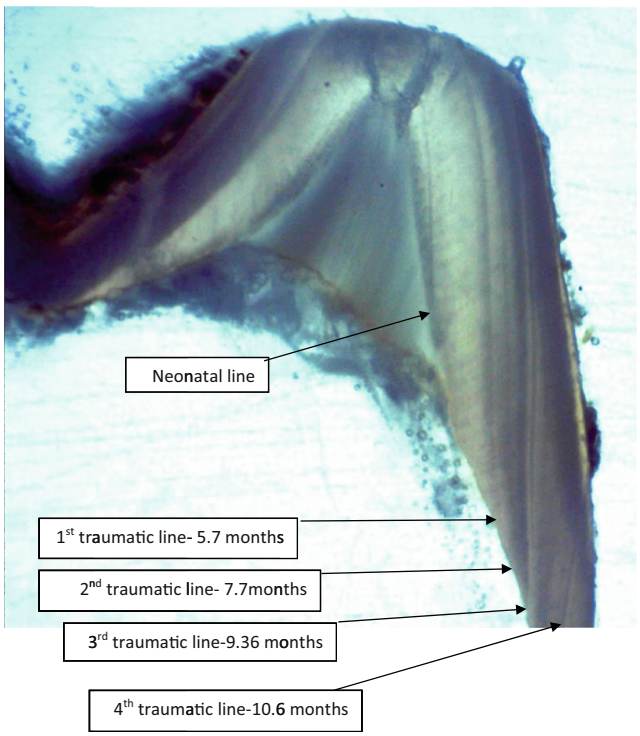

Fig 1 The location of the neonatal line in the second primary molar and the relative timing of the traumatic lines

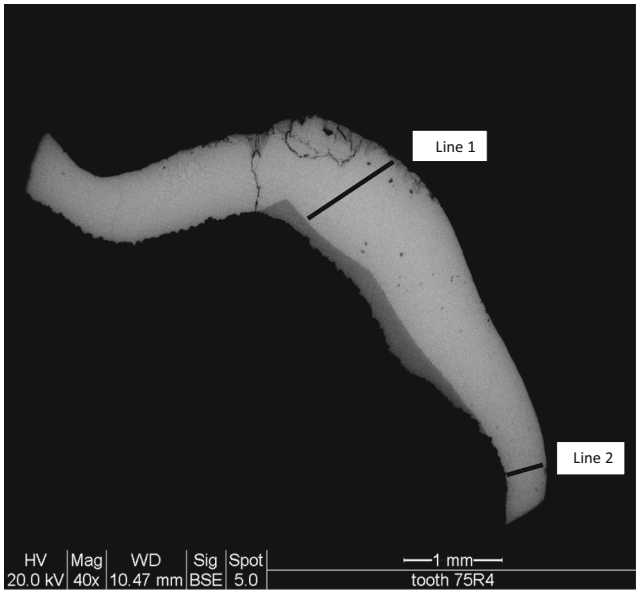

Fig 2 Location of the lines for chemical analyses

Statistical analysis

All data were statistically analyzed using the SPSS 22 package. Independent samples two-tailed Student's *t* test was performed to determine the difference between the elements content for prenatal and postnatal enamel of the control teeth and ITD teeth with $\alpha = 5\ \%$.

Results

Histological examination demonstrated the neonatal and postnatal calico-traumatic lines. Measurements of the distance between the neonatal and calico-traumatic lines correlated with impaired amelogenesis periods at dental ages of 5.7, 7.7, 9.36, and 10.6 months. Figure 1 shows the location of the neonatal line in the second primary molar and the relative timing of the traumatic lines.

Table 2 demonstrates the chemical analysis of the prenatal enamel. The carbon concentration (organic matter) was 3.21 times higher in ITD than in the control group, and the differences were statistically significant. It also contained more magnesium and less phosphate and the differences significant statistically. Calcium level was almost similar between study group and the control, while oxygen level was reduced, but the differences were not significant.

Table 3 shows the chemical analysis of the postnatal enamel. The carbon concentration (organic matter) was 2.37 times higher in ITD (p value = 0.00001), magnesium levels were higher (p value = 0.00001), and phosphate levels were

Table 2- The chemical analysis of prenatal enamel

		N of points	Mean (%)	SD	*p* value
C	1	2637	1.71	1.5	0.000*
	2	1533	5.50	3.9	
O	1	2637	6.92	1.6	0.072
	2	1533	5.92	2.6	
Mg	1	2637	1.63	0.6	0.000*
	2	1533	1.99	1.5	
P	1	2637	35.40	2.6	0.000*
	2	1533	31.92	5.0	
Ca	1	2637	54.34	3.0	0.059
	2	1533	54.65	5.7	

1 = normal, 2 = ITD

*p value < 0.05

reduced (p value = 0.00001). Calcium levels tended to be higher in ITD teeth (p value = 0.059). The Ca/P mean ratio in control teeth (1.552) was lower than the mean ratio in ITD (1.779).

As the finding that carbon concentration in the prenatal enamel was 3.21 times higher in ITD was unexpected, we searched the patient's medical history and found that his mother, a vegetarian, suffered from ulcerative colitis and was hospitalized due to anemia and hypoalbuminemia during pregnancy.

Discussion

Histological analysis of the neonatal line and calico-traumatic lines can reveal clinical and sub-clinical physiological disturbances and negative events that impair the process of amelogenesis. Dating those disturbances is possible by measuring the distance between the neonatal and calico-traumatic lines. The chemical and histological analysis enabled us to confirm that thiamine deficiency in infancy impaired postnatal amelogenesis. The finding that the prenatal enamel was less calcified than the control led us asses in retrospect the mother's medical situation during pregnancy.

Table 3- The chemical analysis of postnatal enamel

		N of points	Mean (%)	SD	*p* value
C	1	3290	1.83	1.3	0.000*
	2	4160	4.34	2.9	
O	1	3290	6.73	1.5	0.063
	2	4160	5.64	2.4	
Mg	1	3290	1.54	0.6	0.000*
	2	4160	1.92	1.4	
P	1	3290	35.41	2.6	0.000*
	2	4160	32.44	4.9	
Ca	1	3290	54.49	3.0	0.048*
	2	4160	55.66	5.4	

1 = normal, 2 = ITD

*p value < 0.05

The higher postnatal enamel carbon concentration found in ITD may derive from either impaired mineralization caused by thiamine deficiency and disturbed cellular metabolism or indirect damage to the ameloblasts due to the physical condition of the patient (encephalopathy, 1.5 months of vomiting, etc.). ITD teeth contained also significantly higher magnesium and lower phosphate concentrations. The higher organic content can be explained by a reduced activity of proteinases and reduced mineralization.

The prenatal stress caused from the mother's medical situation may explain the impaired mineralization of the prenatal enamel. It may also have affected the phosphate concentration in the enamel and caused magnesium levels to rise.

Magnesium concentrations were higher than in the control group, both in ITD pre and postnatal enamel. Calcium and magnesium share similar chemical characteristic and thereby compete during hydroxyapatite formation. Spices, nuts, cereals, cocoa, and vegetables are rich sources of magnesium. Green leafy vegetables are also rich in magnesium since magnesium is required to synthesize chlorophyll. As a vegetarian, the mother's diet might have contributed to a high magnesium level in mother and child's blood, biasing prenatal hydroxyapatite formation to the magnesium-rich compound found in this study.

The amelogenesis of ITD teeth differed from that of the control group. The fact that the Ca/P mean ratio in ITD teeth was higher than the mean ratio in the control shows a damaged mineralization process, expressed also by higher organic contents of control enamel and lower phosphate rate. Calcium can react with other ions such as carbon and form calcium carbonate molecules that will precipitate into enamel. For hydroxyapatite formation, phosphate is necessary, and its low concentration in ITD teeth will cause other calcium components formation instead of hydroxyapatite [17].

The distances between the neonatal and calico-traumatic lines were correlated with the patient's medical history: the first and most prominent calico-traumatic line matched the acute phase of thiamine deficiency that started when the patient was 5.74 months and started vomiting. The second line matched the time he started getting thiamine injections after the outbreak of the issue. The two other lines did not match any documented clinical event, but there may have been subclinical events until the patient reached the ideal nutritional state (thiamine level was never re-checked in the injured infants by any official authority).

The association found in the present work between thiamine deficiency and calico-traumatic lines may point to the role of thiamine in enamel calcification, and further studies are needed to establish the mechanisms involved in this process.

Conclusions

Infantile thiamine deficiency impaired amelogenesis process in primary dentition and resulted in less calcified enamel with a higher level of organic matter. Measuring the location where postnatal traumatic lines meets the DEJ can give us a very accurate estimation of amelogenesis insults that can be correlated with clinical findings.

Compliance with ethical standards

Funding This study was funded by the authors.

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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