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Morphological aspects of dental hard tissues in primary teeth from preterm infants

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Background. Preterm children with very low birth weight suffer from several neonatal and post-natal complications that may affect the mineralization of the teeth. Clinical studies have shown enamel aberrations in both dentitions.

Aims. The aims of this study were to describe enamel histo-morphology in primary teeth, and investigate the relationship between medical history and morphological appearance.

Design. Dental enamels in 44 exfoliated primary teeth, from 14 children with a gestational age below 29 weeks and with a very low birth weight, were investigated, using polarized light microscopy (POLMI) and under a scanning electron microscope (SEM).

Results. The neonatal line was found in 1/3 of the sections located coronally of the crown. In the post-natal enamel, 31 teeth showed a degree of porosity higher than 5% with a varying extension. More than half of the teeth showed one or more increment lines. The SEM analysis confirmed the POLMI findings with irregular prisms covered with a structure-less film.

Conclusions. Enamel from primary teeth of preterm children was found to have a high frequency of mineralization disturbances found in POLMI and SEM. The morphological features of the enamel from preterm children do not reflect the disturbances on general growth and development occurred during the neonatal period.

Introduction

The survival rate of extremely preterm children [< 25 weeks + 6 days gestational age (GA)] is much higher today. The frequency of preterm children has not changed, but the prognoses of survival of these infants have increased[1,2]. Preterm children with a very low birth weight (VLBW ≤ 1500 g) or extremely low birth weight (ELBW < 1000 g) often suffer from complications such as hyperbilirubinemia; perinatal asphyxia; respiratory, cardiovascular, neurological and nutritional deficiencies; infections; and/or gastrointestinal problems. Complications are more common in cases with a low GA and a low birth weight[1,2]. All of these complications have been reported to be aetiological factors behind the disturbed mineralization in primary teeth. An increased prevalence of mineralization disturbances in primary teeth has been observed in preterm children, that is, < 37 gestational weeks[3–7].

The disturbed calcium metabolism during the first days of life may be an important factor behind enamel aberrations in the primary teeth as well as the fact that the major accumulation of calcium and phosphate takes place during the last trimester of pregnancy[8]. The earlier a child is born, the less calcium and phosphate are accumulated. The effect of disturbed calcium metabolism on the mineralization of teeth appears to depend also on other post-natal complications[3,8,9]. Early vitamin D

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and mineral supplementation has not reduced the frequency of clinically visible mineralization disturbances in the primary teeth. In addition to systemic factors, trauma caused by a laryngoscope and endotracheal intubation also seems to be associated with mineralization disturbances in preterm children[10,11].

Primary teeth start their development during pregnancy and are completed in early childhood. The mineralization of primary teeth starts during the fourth month of pregnancy, and crown completion has occurred by 1 year of age. There are several factors that interfere with tooth formation, presented in literature. Prenatal factors associated with mineralization disturbances are maternal infections, metabolic diseases, nutritional disorders, and maternal intake of substances such as tetracycline and thalidomide. Perinatal and post-natal factors associated with mineralization disturbances are birth complications, post-natal infections, metabolic diseases, congenital cardiac diseases, gastrointestinal malabsorption, nephritic syndrome, chronic renal failure, and biliary atresia; nutritional disturbances and the intake of substances such as tetracycline, lead, and fluoride[3,7,9,11].

Enamel defects are basically classified under two main categories: enamel hypoplasia and enamel hypomineralization. Whereas enamel hypomineralization is a qualitative defect, identified visually as a change of translucency of the enamel, enamel hypoplasia is a quantitative defect. Enamel hypoplasia tends to be more frequent in preterm children than the hypomineralization in primary teeth[6,12].

The morphology and chemical composition of primary teeth from preterm children and children with LBW or VLBW have previously been studied in a polarized light microscope, scanning electron microscope (SEM), X-ray microtomographic, secondary ion mass spectrometry (SIMS), and by ion probe technique[13–16]. Those studies have shown incomplete enamel maturation, especially cervically and subsurface lesions (SSLs) in the primary teeth from preterm children, and enamel hypoplasia were found frequently. It has been shown that enamel in primary teeth from preterm children, with VLBW examined in SEM, was thinner and had more hypoplastic changes than enamel from full-term children[15,16]. However, there are few studies combining polarized light microscopy (POLMI) and SEM. By combining polarization microscopy with SEM, the enamel may be examined both on a light microscopical and ultra-structural level, whereby more information may be gained.

The aim of this study was to investigate the morphological appearance of primary enamel from preterm infants, utilizing POLMI and scanning electron microscopy, and comparing the results with neonatal medical data.

Materials and methods

Patients

Forty-five patients born before a GA of 29 weeks, during the years 1988–1991, at Östra Sjukhuset in Göteborg, Sweden, were invited to participate in the study. The patients had earlier participated in clinical ophthalmologic and growth studies[17–19]. Informed consent was obtained from all the patients and their parents. The patients were asked to send exfoliated or extracted primary or permanent teeth in a prepaid envelope. Fourteen patients accepted (Table 1). The reason for not participating in the study was that exfoliated teeth were not saved.

Tooth material

Exfoliated or extracted primary teeth (44 teeth: 20 incisors, 4 canines, 20 molars) and one permanent tooth (not included in this study) were collected (Table 2). All teeth were macroscopically examined for opacities and enamel hypoplasias. The teeth were embedded in an epoxy resin (Epofix, Electron Microscopy Sciences, Fort Washington, PA, USA). Sagittal longitudinal serial sections (thickness of approximately 100 μm) were prepared in a Leitz low-speed saw microtome (Leitz, Wetzlar, Germany)[20].

Medical data

The patients' neonatal and post-natal medical history was retrieved from the hospital and is given for 14 children in Table 1.

Table 1. The occurrence of post-natal complications and treatments among the study patients.

Patient no.	IRDS	HEM	HB	BT	AV	CPAP	O_2d
1	Yes	Yes	No	4 + 1	0	7	62
2	Yes	Yes	Yes	5 + 1	0	6	41
3	Yes	Yes	No	21 + 22	35	60	184
4	No	No	No	No	6	54	53
5	Yes	No	No	45 + 39	61	37	471
6	Yes	Yes	Yes	No	0	2	37
7	No	No	No	1	0	3	2
8	No	No	Yes	3 + 2	0	10	55
9	Yes	No	No	5	17	17	38
10	No	No	No	1 + 1	0	2	15
11	No	No	Yes	3	0	3	12
12	Yes	No	Yes	2	0	3	3
13	Yes	No	No	4	0	12	62
14	No	No	Yes	No	0	0	2

AV, number of days with artificial ventilation; BT, number of blood and plasma transfusion; CPAP, number of days with nasal oxygen with overpressure; HB, hyperbilirubinemia; HEM, haematologic infection; IRDS, infant respiratory distress syndrome; O_2d, number of days with only oxygen treatment.

Table 2. Demographic and dental data.

Patient no.	GA	BW	sdsW	Tooth
1	28.14	1130	–0.91	1* M
2	28.86	1370	–0.19	1* I. 1*M
3	26.00	690	–2.22	1* I. 1*M
4	28.86	1015	–2.36	1* I
5	25.86	695	–2.01	1* I. 1*M
6	27.86	1280	0.25	6* I. 1*C. 3*M
7	28.71	1250	–1.02	3* M
8	27.43	1180	0.09	1* I
9	28.57	1450	0.48	2* M
10	28.14	1140	–1.14	1* I. 1*C
11	28.14	1180	–0.67	1* I. 1*M
12	27.00	690	1.49	6* I. 2*C. 4*M
13	27.00	1000	–0.65	1* M
14	28.00	1425	0.89	1* I. 2*M
Mean	27.76	1107		
SD	0.98	261		

Gestational age in weeks (GA), birth weight in grams (BW), standard deviation score of BW (sdsW), and number of collected primary teeth from the preterm children (X* = number of teeth, I = incisor, C = canine, M = molar).

Polarized light microscopy (POLMI)

In all sections, enamel was examined in polarized light, both dry in air and after water imbibition, in an Olympus (Tokyo, Japan) polarizing microscope. Polarization microscopy has been used in numerous studies of dental enamel; therefore, only a short description will be given[14,21–26]. In POLMI, normal mineralized enamel appears negatively birefringent when examined in dry air, whereas less well-mineralized enamel appears with a positive birefringence. When examined, positive birefringent enamel still remains positive after water imbibition, indicating that the enamel has a microporosity of more than 5%. However, if it changes to negative birefringent, the degree of porosity is less than 5%. When the enamel has negative birefringence in the post-natal enamel, before and after water imbibition, it is regarded as having a normal degree of mineralization. Micro-photos were taken of the central sections, which were used for the SEM analysis.

The extent of the positive birefringence in the post-natal enamel when examined dry in air was registered in three groups: 1 (when positive birefringence is noted in the surface region only), 2 (when the extent is <1/2 of the enamel thickness), and 3 (when the extent is > 1/2 of the enamel thickness). The extent of the positive birefringence in the enamel after water imbibition (> 5% degree of porosity) was registered as: 0-wi (when the degree of porosity is < 5% in the enamel after imbibition), 1-wi (when positive birefringence is seen only as streaks), 2-wi (when the extent is < 1/2 of the enamel thickness), and 3-wi (when the extent is > 1/2 of the enamel thickness).

The localization of the microporous zone was registered by dividing the tooth into five groups: A (< 1/4 of the crown coronally), B (1/2

of the crown coronally), C (2/3 of the tooth coronally), D (the central part of the tooth), and E (the total crown).

The neonatal line (NNL) and increment lines were registered both in dry air and after water imbibition. An SSL was seen as a thin positive birefringent subsurface zone, registered in dry air and after water imbibition.

The POLMI examination was carried out in three steps by two of the authors (MR, JGN). In the first step, all specimens were examined by both together, whereafter each examiner examined the sections, and in the third step the findings were compared and any differences were discussed.

Scanning electron microscopy (SEM)

Four sections representing four patients were, after the POLMI analysis, mounted on sample holders for SEM with carbon tape. The four teeth were selected in the POLMI analysis having varying degrees and extent of birefringence > 5% after water imbibition. The sections were etched for 30 s with 30% phosphoric acid, and carefully rinsed with de-ionized water. For the SEM analysis, the sections were sputter coated with gold by vapour deposition. The SEM examinations were carried out in a Philips SEM 515 at 20 kV (Philips, Eindhoven, The Netherlands). The sections were also analysed in a field emission SEM (Gemini IMB, LEO 1530, Oberkochen, Germany). The analyses were carried out at 20 kV in three locations in hypomineralized and normal enamel, close to the surface, middle enamel, and close to the enamel dentin junction.

Ethical aspects

Ethical consent was given by the Ethical Research Committee at Göteborg University, Dnr: 262-06.

Results

Macroscopical examination

The examined teeth appeared to have a marked abrasion, however, not apparently more than normal. In only one tooth, an enamel hypoplasia was found.

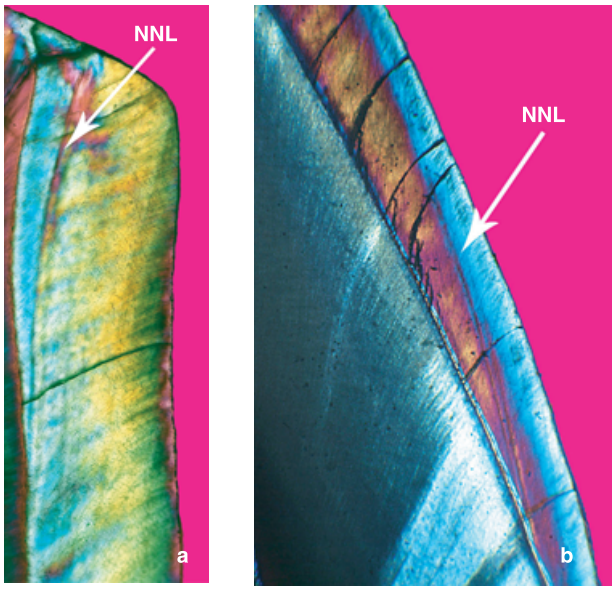

Fig. 1. (a) Un-decalcified section of a primary incisor from a preterm infant, seen dry in air in polarized light with a neonatal line (NNL). Original magnification ×40.
(b) Un-decalcified section of a primary incisor from an infant with normal gestational age seen dry in air in polarized light with an NNL. Original magnification ×40.

POLMI analysis

The data of the preterm infants are given in Tables 1–2, and the morphological findings from the POLMI analysis are given in Table 3. The presence of an NNL made it possible to discriminate prenatal enamel from post-natally formed enamel in 17 of the teeth (15 incisors, 1 molar, and 1 canine). The NNL was identified by its location in relation to tooth type and gestation age; further, the NNL is more distinct than other incremental lines. The NNL was not found in 27 of the teeth (19 molars, 3 canines, and 5 incisors). When the sections were examined in dry air, the NNL appeared positively birefringent, extending from the enamel dentin junction coronal to the middle of the crown in all incisors, to the surface of the enamel (Fig. 1a). Water imbibition did not change the positive birefringence of the NNL. The location of the NNL was more incisally located compared with that seen in primary enamel from normal full-term infants (Fig. 1b). This is in concordance with the short GA of the patients (Table 2).

The prenatal enamel appeared positively birefringent in all teeth examined dry in air,

Table 3. The morphological findings from the polarized light microscopy analysis and data of the pre-term infants.

Patient no.	Tooth	NNL	L	Pren	Postn	IMb	SSL	INC
1	SM	N			3C	3-wiC		
2	LI	Y	2	X	3D	2-wiD		
	SM	N			3E	3-wiD	X	
3	UI	Y	3		3B	2-wiA	X	X
	SM	N			3C	0-wi		X
4	UI	N			3B	2-wiB		X
5	UI	N			N	N		
	FM	N			2B	0-wi		
6	UI	Y	2		3C	1-wiD		X
	UI	Y	2	X	3E	2-wiB		X
	C	Y	4		N	N		X
	LI	Y	4		N	N		
	UI	N			3C	3-wiB		X
	LI	Y	4		2B	0-wi		X
	LI	Y	3		2A	2-wiA	X	X
	SM	N			3C	2-wiC	X	X
	SM	N			3C	3-wiB	X	X
	FM	N			3C	3-wiC		X
7	SM	N			3C	3-wiC		X
	SM	N			3C	3-wiC	X	
	SM	N			3E	3-wiC		
8	UI	N			3C	3-wiC		
9	FM	N			3B	3-wiA	X	X
	SM	Y	4		3B	2-wiA		
10	C	N			3C	3-wiB	X	X
	UI	Y	3	X	3C	0-wi	X	X
11	FM	N			3E	0-wi	X	X
	UI	Y	3	X	3E	0-wi	X	X
12	UI	Y	3		N	N		
	UI	Y	3	X	2A	0-wi		X
	UI	Y	2		3C	0-wi		
	C	N			3C	1-wiC	X	X
	FM	N			3C	1-wiC	X	X
	SM	N			3C	1-wiD	X	X
	SM	N			3C	3-wiC	X	
	LI	Y	2		N	N	X	X
	LI	Y	3		3B	1-wiD		X
	UI	N			3B	2-wiD		
	C	N			3B	2-wiB	X	X
	FM	N			3C	3-wiC	X	X
13	FM	N			3C	3-wiC		
14	FM	N			3C	1-wiD		X
	LI	Y	2	X	3A	1-wiA	X	X
	FM	N			3C	1-wiD		

Tooth: C, canine; FM, first molar; LI, lower incisor; SM, second molar; UI, upper incisor.
NNL, neonatal line: N, not found; Y, present.
L, localization of the NNL: 1, cervical 1/3; 2, middle 1/3; 3, incisal 1/3; 4, cuspal/incisal.
Pren, prenatal enamel, after water imbibition: X, with > 5% degree of porosity.
Postn, post-natal enamel examined dry in air: N, normal; 1, positive birefringence at the enamel surface; 2, positive birefringence < 1/2 of the enamel; 3, positive birefringence > 1/2 of the enamel; A, < 1/4 of crown; B, 1/2 of crown; C, 2/3 of crown; D, central; E, entire crown.
IMB, post-natal enamel after water imbibition: N, normal; 0-wi, < 5% degree of porosity; 1-wi, streaks of > 5% degree of porosity; 2-wi, > 5% degree of porosity < 1/2 of the enamel; 3-wi, > 5% degree of porosity > 1/2 of the enamel; A, < 1/4 of crown; B, 1/2 of crown; C, 2/3 of crown; D, central; SSL, subsurface lesion; X, present.
INC, increment lines: X, present.

seen as an inner micro-porous zone (Fig. 2a). After water imbibition, six teeth remained positively birefringent indicating a degree of porosity of more than 5% (Fig. 2b).

When the post-natal enamel was examined dry in air, it appeared negatively birefringent in five teeth (four incisors and one canine). The remaining 39 teeth exhibited a positive

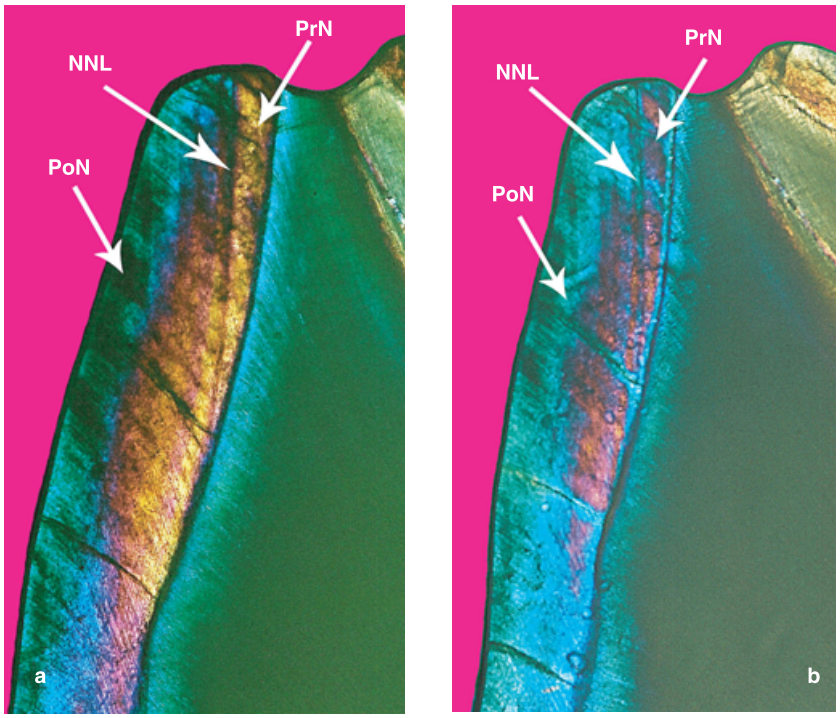

Fig. 2. (a) Un-decalcified section of a primary molar from a preterm infant, seen dry in air in polarized light with a neonatal line (NNL) and prenatal porous zone (PreN) (PostN = post-natal enamel). Original magnification ×40. (b) Un-decalcified section of the same primary molar as in (a), seen after water imbibition in polarized light with an NNL and PreN (PostN = post-natal enamel). Original magnification ×40.

birefringence of varying extension in the enamel (Fig. 3a). Most of the positively birefringent enamel changed to negative birefringence after water imbibition. The central parts remained with a positively birefringent appearance, indicating a pore volume distribution of more than 5% (Fig. 3b).

Eight teeth from the sections with initial positive birefringence showed a negative birefringence after water imbibition, five of these being incisors. The remaining 31 teeth changed partly to negative birefringence, thus showing various degrees and extensions of porous enamel. In eight of these 31 teeth, the micro-porous zone gave an impression of streaks with varying degrees of porosity in the enamel (Fig. 3c). In 26 teeth (17 molars, 7 incisors, and 2 canines), a positive birefringence extending over more than 2/3 of the crown was noted when examined dry in air (Fig. 3d). Twenty-one of these sections remained positively birefringent after water imbibition, indicating a degree of porosity of more than 5%.

SSLs were found in the palatal and buccal enamel in 19 teeth, twice as often in molars as in incisors and in three of four canines (Fig. 4a). The lesion was always located in the post-natal enamel and was seen as a thin micro-porous zone under a well-mineralized surface. SSLs were more often seen in teeth with a microporous zone > 5%, but also seen in teeth with normal enamel.

No connection or relation with other structural changes in the enamel could be found.

Prenatal incremental lines were found only in one tooth, whereas one or more post-natally located incremental lines were found in 28 teeth (Fig. 4b). All incremental lines appeared positively birefringent both dry in air and after water imbibition. No connection between the microporosities and the recorded increment lines could be found.

SEM analysis

The SEM analysis showed that the hypomineralized enamel, as seen in POLMI, differed from normal enamel, with a less regular orientation of prisms and a difference in the overall appearance of the prisms in the hypomineralized areas. In normal enamel, the prisms were readily seen. In the hypomineralized enamel, however, the prisms appeared to be covered with structure-less film, indicating remains of organic matter (Fig. 5a,b).

The incremental lines were seen as a wavy structure in the enamel (Fig. 5c,d). Because the

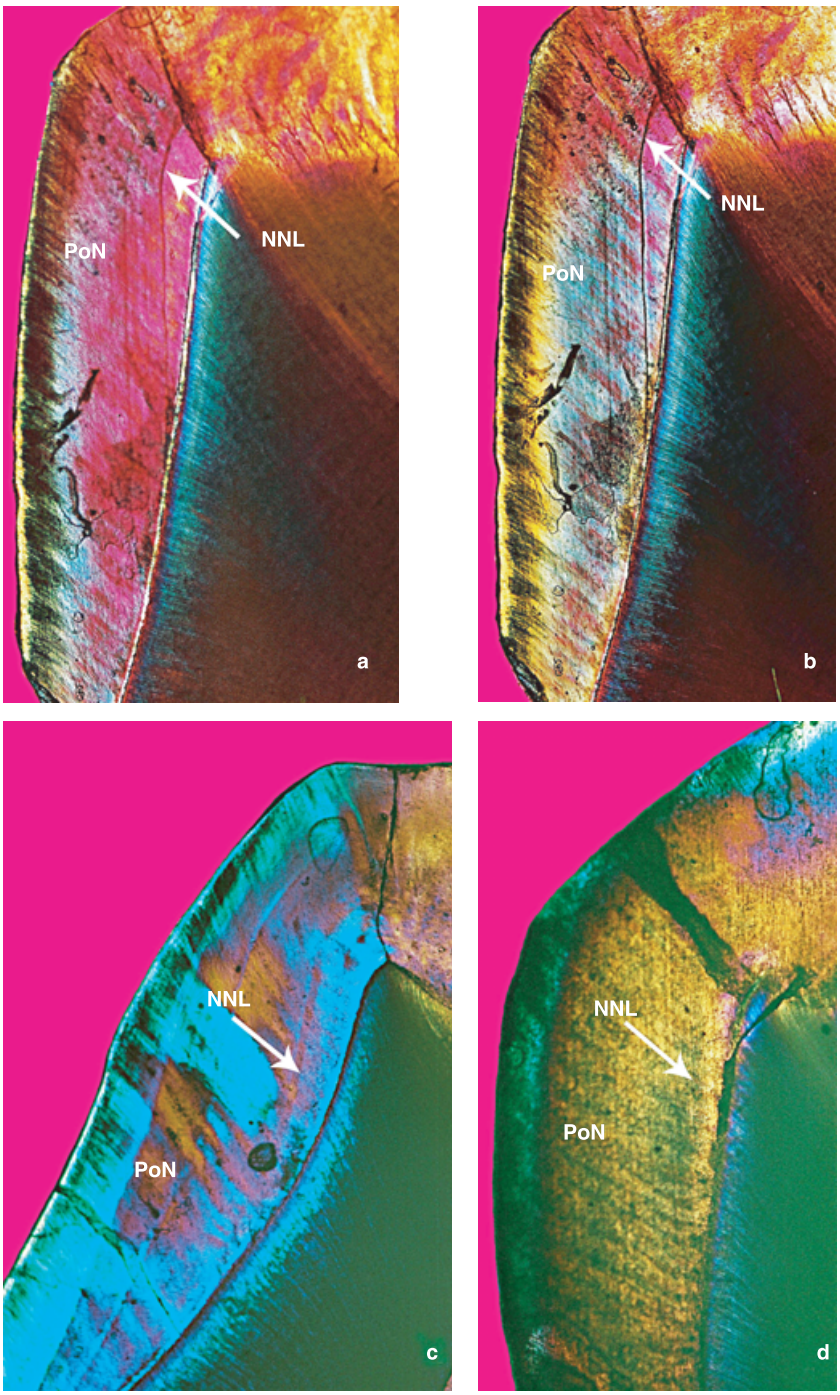

Fig. 3. (a) Un-decalcified section of a primary molar from a preterm infant, seen dry in air in polarized light with a neonatal line (NNL) and hypomineralized post-natal enamel (PostN) (PreN = prenatal enamel). Original magnification ×40. (b) Un-decalcified section of a primary molar from a preterm infant, seen after water imbibition in polarized light with an NNL and hypomineralized post-natal enamel (PostN) (PreN = prenatal enamel). Original magnification ×40. (c) Un-decalcified section of a primary molar from a preterm infant, after water imbibition in polarized light with an NNL and post-natal enamel with varying degrees of hypomineralization (PostN) (PreN = prenatal enamel). Original magnification ×40. (d) Un-decalcified section of a primary molar from a preterm infant, seen dry in air in polarized light with an NNL and post-natal enamel with severe hypomineralization of the enamel (PostN) (PreN = prenatal enamel). Original magnification ×40.

incremental lines most often appeared in hypomineralized areas, the enamel showed a less distinct structure as compared with normal enamel.

When the results from the morphological analysis were compared with the patient's medical charts, there was no evidence that the severity of the aberrations in enamel was related to the patient's medical history (Tables 1–3).

Discussion

This study has shown that histo-morphological studies of teeth from early preterm children displayed a variety of changes in the enamel. The main findings were an increased porosity with more than 5% pore volume, especially in molars and canines. Because the number of teeth in this study was collected from only 14

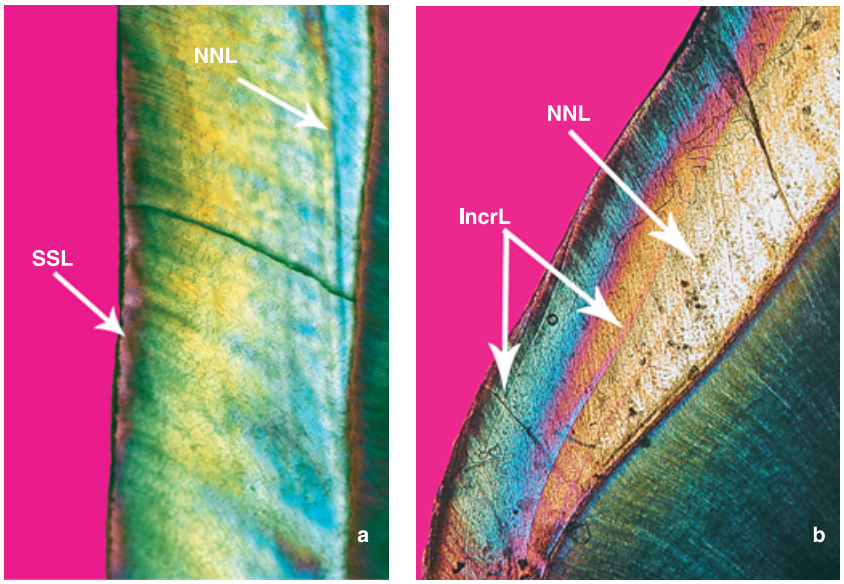

Fig. 4. (a) Un-decalcified section of a primary incisor from a preterm infant, seen dry in air in polarized light with a neonatal line (NNL) and subsurface lesions (SSLs) in the post-natal enamel. Original magnification ×40.
(b) Un-decalcified section of a primary molar from a preterm infant, seen dry in air in polarized light with an NNL and incremental lines in the post-natal enamel (IncrL). Original magnification ×40.

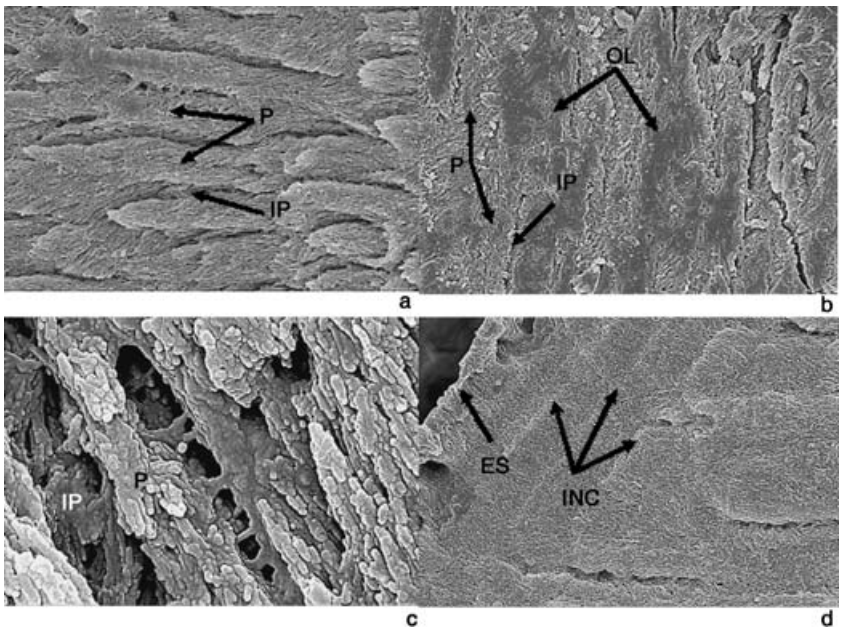

Fig. 5. (a) Un-decalcified section of a primary incisor seen in scanning electron microscope (SEM) with normal enamel (P = enamel prism; IP = interprismatic area). Magnification ×3000.
(b) Un-decalcified section of a primary incisor from a preterm infant, seen in SEM with hypomineralized enamel covered with a structure-less 'film' (P = enamel prism; IP = interprismatic area; OL = organic layer on the prisms – the structure-less film). Magnification ×3000. (c) Un-decalcified section of a primary incisor from a preterm infant, seen in SEM in high magnification of the porous hypomineralized enamel (P = enamel prism; IP = interprismatic area). Magnification ×30 000.
(d) Un-decalcified section of a primary molar from a preterm infant, seen in SEM showing the enamel structure in an area of incremental lines (ES = enamel surface; INC = incremental lines). Magnification ×3000.

children, and 22 of the teeth came from two of the children, conclusions concerning the effect of neonatal complications should be made carefully.

One drawback is the lack of matched control teeth; however, the possibilities to collect such teeth were limited, and primary teeth from only two patients were collected. However, the results were compared with results from previous histo-morphological studies of enamel in primary teeth from normal full-term infants carried at the department[14].

The techniques used for the histological examination in this study have previously been used for investigations of the enamel structure of primary and permanent teeth, and may therefore be regarded as established and well-known methods[14,21–26].

In most of the sections, mainly molars, no prenatal enamel could be seen, which is explained by the short GA of the infants (< 29 weeks). Accordingly, the NNL was found more incisally in the incisors. However, because of normal attrition of the primary incisors, the NNL was not always discernable. Where the NNL could be observed in the sections, it was distinct.

The overall morphological appearance of the prenatal enamel did not differ from what has been described earlier for normal enamel[21,24,25,27,28]. Some teeth appeared to have a more hypomineralized character. However, because the amount of prenatal enamel was very limited because of the short GA and attrition, no conclusions can be made.

In preterm children, the major part of the enamel is mineralized after birth and may thus be subjected to numerous factors which might disturb the mineralization. The post-natal enamel had partly a zone of hypomineralized enamel that may be attributed to disturbances in calcium metabolism. This is in concordance with a previous histological study of enamel in primary teeth from low-birth-weight infants, where an increase of hypomineralized post-natal enamel was found[14]. In a light microscopic and scanning electron microscopic investigation of teeth from VLBW infants, it was shown that the enamel visually was significantly thinner compared with normal control children. Further, the authors also found a high frequency of enamel hypoplasias[16]. No measurements of the thickness of the enamel were carried out in this study because the teeth were abraded to such an extent that it would not be feasible. The hypomineralized character of the enamel was confirmed by the SEM examinations. The findings of a less distinct prism structure are supported by SEM studies of hypomineralized enamel in first permanent molars and primary teeth, from patients with 22q11 deletion syndrome[22,23,29].

Hypoxia, hypocalcemia, as well as intubation are known to cause enamel hypoplasia, and preterm infants often are intubated; it could be expected to be found more frequently[10,11]. However, only one tooth with an enamel hypoplasia was found in this material, which is in contrast to what has been described in studies of primary teeth from VLBW infants[16]. The reason for this might be few teeth in the study emanating from few patients. Only four patients had been intubated.

The findings of so-called SSLs in the post-natal enamel have been described in previous articles and have been found in primary enamel from children born within a normal gestational length and in low-birth-weight infants[14,16]. Their presence in the primary enamel in both incisors and molars of the preterm infants may therefore not be related to their short GA because the mineralization of the enamel in the location of the SSL takes place long after birth[21].

The presence of incremental lines in the enamel has been suggested to be a response to disturbances in the calcium metabolism and the following hypocalcemic state, much in the same way as the presence of the NNL is explained[14,27,28]. The preterm infants in this study have suffered from several perinatal complications such as severe asphyxia, infections, blood transfusions, elevated B-glucose values, hyperbilirubinemia, and IRDS, as well as treatment in respirators and CPAP for different periods of time. In this study, however, these severe conditions were not reflected as increased increment or in any major morphological aberrations in the primary enamel. The fact that the number of teeth in this study was low and from few patients makes it difficult to conclude that the morphological features of the enamel from preterm children do not reflect the disturbances on general growth and development occurred during the neonatal period. This calls for more studies on the subject including further studies of the dentin and also utilizing other methods.

It may be concluded that enamel disturbances, such as hypomineralized areas and incremental lines in the post-natal enamel, are more frequent in primary teeth from preterm children with very low GAs.

Acknowledgements

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What this paper adds

- This paper describes the histo-morphology of enamel in primary teeth from preterm-born children with a GA of less than 29 weeks, and with a very low/extremely low birth weight.
- The post-natal enamel exhibits a hypomineralized zone which may also be found in SEM examinations.
- The morphological aberrations found in the post-natal enamel are apparently not caused by one single medical condition.

Why this paper is important to paediatric dentists

- It is important to understand that enamel in primary teeth from preterm children is of a lower quality.
- It gives a deeper understanding of the effects of neonatal medical conditions on the mineralization of primary teeth.

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