Deciduous neonatal line: width is associated with duration of delivery

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Abstract

The delivery-related neonatal line (NNL) appears into the enamel of primary teeth and first permanent molars at birth and is a marker of live birth process. It varies in width and its location, is different in each deciduous tooth type, and is indicative of gestation time. It is unclear which triggers determine NNL at birth. Our objective was to investigate the effect of the duration and mode of delivery on NNL width.

NNL of 129 teeth, a collection derived from a long-term, prospectively followed population cohort, was measured under light microscope. Altogether, 54 sections with most optimal plane of sectioning were analysed for the duration and mode of delivery.

NNL was detected in 98 % of the deciduous teeth with the median width of 9.63 µm (min 3.16 µm, max 27.58 µm). A prolonged duration of vaginal delivery was highly significantly associated with a narrower NNL ($r = -0.41$, $p = 0.0097$). No significant association was found between the width of NNL and mode of delivery ($p=0.36$).

NNL is demonstrable in virtually all deciduous teeth. The width seems to be inversely proportional to the duration of delivery. Causes of the inverse proportion are speculated to result from altered amelogenesis induced by prolonged and intensified delivery-associated stress. Further research is needed to clarify the underlying mechanisms.

Keywords:

neonatal line
forensic odontology
long-term study
mode of delivery
duration of delivery
population sample

1. Introduction

Embryonal tooth enamel development starts in about the 10th week of pregnancy. In a circadian rhythm, appositional layers of organic enamel matrix are formed. Mineralization of the matrix, where hydroxyapatite units form alongside, wavely running enamel prisms, is initiated soon after matrix secretion giving mature enamel an `onion-like´ appearance [1]. At birth, a particularly well discernible layer, called neonatal line (NNL), is formed. NNL was first named in 1936 by Schour [2] who described it as"a distinctive incremental line in the enamel and a corresponding incremental line in the dentin". NNL is found in the dentition which is in active development at birth: in all deciduous teeth and sometimes in a mesiobuccal cusp of the first permanent molar [3]. NNL separates pre- and postnatal enamel and dentin and varies in location in different tooth types [2]. The thickness of prenatal enamel gradually grows from preterm to post-term and, consequently, the location of NLL changes [4]. NNL makes up one measure of prenatal and postnatal development of a child. The presence and characteristics of NNL are particularly important in forensic medicine, in alleged infanticide with decomposed human remains, because NNL is an evidence of live birth.

Under light microscope, NNL is visible as a dark, sharpish band against the surrounding lighter enamel. In microradiograph analyses, both Weber & Eisenmann [5] and Sabel et al

[6] showed hypo mineralization in NNL. Maturation of enamel in NNL may continue to occur after it has formed, ending as equally mineralized as the surrounding enamel [7]. This continual maturation can partly explain why NNL is not seen with its total length [6]. The line is usually slightly wider in the middle third of the crown length [6] because the secretion rate of ameloblasts is bigger in this area of the crown wall [8]. Diffuse line was explained by Weber & Eisenmann [5] to result from oblique cutting. The width of NNL is reportedly from few up to 30 µm [5, 9, 10, 11, 12, 13]. Eli et al [9] demonstrated that, compared to cesarean section, NNL was wider in spontaneous vaginal delivery and still wider in assisted vaginal delivery, even though the width measurements overlapped the adjacent mode of delivery groups. Zanolli et al [10] could not confirm the results of Eli et al. but, in contrast, Canturk et al [11] showed significant ascending average widths in NNLs from cesarean section to spontaneous vaginal delivery.

Data on the effects of the mode of delivery on NNL width are controversial [9, 10, 11]. To our knowledge, no one has investigated the effect of the duration of delivery on the width of NNL. Our purpose was to study and characterize NNL width and associated factors, particularly the effect of duration and mode of delivery on the neonatal line of deciduous teeth in a Finnish random population cohort sample. Our hypothesis was that the duration and mode of delivery affect the width of NNL.

2. Subjects and methods

2.1 Subjects

The subjects were derived from a representative, prospectively followed population cohort study, the Finnish Family Competence study (FFC) [14]. The source population originated from a geographically defined catchment area (joint population of 713.000 in 1986) of the Turku University Hospital in south-western Finland. Data collection was based on stratified randomized cluster sampling. Of 1713 young families expecting their first child and eligible to the study, 1443 gave their informed consent to participate. Sociodemographic, socioeconomic and medical data of the families and their children were prospectively collected, from 10th week and 28th week of pregnancy, at birth and on six visits to well-baby clinics until the age of 7 years. All the diagnoses were documented with ICD-9 codes by public health care professionals. Socioeconomic status was defined according to the criteria of the Central Statistical Office of Finland based on the United Nations recommendations [15]. The baseline and follow-up study designs were approved by the Joint Ethical Review Committee of the University of Turku and Turku University Hospital (DNO 540/582/85).

The parents were requested to deliver the child´s one spontaneously loosened or removed deciduous tooth for research purposes. Teeth were received from 146 of 1285 live-born children in 1986-87. A form attached to the tooth at collection stage categorized it as maxillary/mandibular and left/right. Nine teeth were broken and in seven teeth subject data were defective for the identification of the child. Thus, 129 children´s deciduous teeth were

included in the collection later to be referred to as the Finnish Deciduous Tooth collection (FDT-collection).

Dropout analysis between the 129 participants and 1156 non-participants for socioeconomic and sociodemographic parameters showed no significant differences, except for a higher mean age (1,5 years) ($p<0.001$, t-test) and residence in an urban area ($p<0.001$, Fisher´s Exact Test) of the participants.

2.1.1 Teeth

The teeth were preserved in 100 % alcohol. Samples were run through rising alcohol series (70 %, 90 %, 100 %), embedded in resin (Technovit® /alcohol (50%/50%), 100% Technovit® two times; Heraeus Kulzer, Hanau, Germany). Sections of 20 microns were made by buccal-lingual/palatial and axial cutting, and when no wear appeared, through incisal middle and cusp tip/underlying dentin horn with EXAKT 300 CP Band System and EXAKT 400 CS Micro Grinding System (EXAKT Technologies Inc., Oklahoma City, USA).

2.2 Methods

2.2.1 Structural analysis of neonatal lines

All 129 teeth were studied under light microscope (Leica DM600B with 1x-1,25x-1,6x magnifications attached to Leica DMC2900 camera, objectives 1,25x/0.04, 2,5x/0.07, 5x/0.15, 10x/0.40 and 20x/0.70, Solms, Germany).

The samples were divided into two groups based on the degree of tooth wear (progressive material loss of a tooth's surface): Group A (n=54), samples with no to moderate wear in enamel and with sharp dentin horn. This group included also those with wear through enamel slightly up to dentin but a sharp dentin horn easily detected, to best achieve the optimal plane of sectioning. The ground sections in Group A were accepted for statistical analysis for the duration and mode of delivery. Group B (n = 75) samples included teeth with heavy wear till dentin and were not used for statistical analysis for duration and mode of delivery.

NNL was identified in the whole collection (n = 129) by its location in relation to each tooth type. Besides reported characteristic (variation in width and continuity) three samples had a furrow-like appearance, with two quite even-in-thickness lines, very close to each other and weak difference in distinctiveness.

NNL thickness was measured on one level from buccal/labial surface, in the most possible middle third of the tooth crown wall, with 10x/0.40 focus. From furrow-like appearance, the first line was measured. Six measurements were done inside the focused area with Leica Application LAS X software and the mean was calculated (Fig.1.). Measurements of NNL were done along the prism path in order to best capture the `passed time´. To assess the inter-observer variation of the results, measurements were done by two authors (JH, VV) in 44 teeth (from Group A n = 23 and Group B n = 21).

2.2.2 Statistical analysis

NNL widths between sexes were compared with one-way analysis of variance in all samples (n = 129). Within Group A (n = 54) the same analysis was conducted to show differences in width between delivery modes (cesarean section, spontaneous vaginal and assisted vaginal delivery with the latter including forceps and suction cup). Logarithmic transformation was used to NNL width to achieve normality assumption. Pearson correlation between duration of delivery and line width (log transformed) was calculated. Because two observers did NNL measurements, inter-observer variation was evaluated with Pearson correlation. Significance level of <0.05 (two-tailed) was considered statistically significant. Analyses were performed with SAS® System, Version 9.3 for Windows.

3. Results

3.1 Sociodemographic and socioeconomic background

The mean age of mothers (n = 129) at delivery was 27 years (SD 4.36, median 27, range 17–38 years). The mothers had no long-term (>6 months) diseases, except for two who were diagnosed with hypothyroidism and hyperthyroidism, respectively. Of the mothers, 121 lived in urban and 8 in rural areas. Neither socio-economic status nor boy/girl distribution of children were significantly different from the general population (p=0.14). Table 1 shows characteristics of the FDT collection.

3.2 Neonatal line width

NNL was recognized in 98 % (126/129) of the deciduous teeth. Three (2 %) teeth with absent NNL represented all deciduous tooth types (incisor, canine and molar). Median widths of NNL varied in different groups (Table 2). The Pearson correlation coefficient in the inter-observer variation was 0.95. The NNL widh did not significantly differ between boys and girls (p = 0.39).

3.3 Neonatal line width and duration of delivery

In teeth with no to moderate wear (Group A) a highly significant correlation between NNL width and duration of vaginal deliveries (spontaneous n = 28, assisted n = 11) was observed: the longer the duration of delivery the narrower the NNL (Table 3). Duration of delivery is defined to start when contractions appear in five minute intervals and to finish at birth.

3.4 Neonatal line width and mode of delivery

NNL width was not significantly associated with the mode of delivery (p = 0.36). There was a rise of median NNL width in spontaneous vaginal deliveries vs. assisted vaginal deliveries but the median in cesarean sections settled in-between (Table 4), and there was overlapping in width measurements across all modes of delivery.

4. Discussion

The neonatal line was recognized in virtually all deciduous teeth and all the measured values of NNL width are in agreement with previous studies [5, 6, 9, 10, 11, 12, 13]. Interestingly, in the present study, NNL width was inversely proportional to the duration of delivery. Accordingly, a prolonged delivery process might inhibit the development of NNL. So far, factors affecting amelogenesis in the formation of NNL and the variation in width are largely unknown [5, 6, 16]. Schour [2] suggested that ”Neonatal lines are caused by metabolic changes resulting from the experience that the infant undergoes at birth and during its neonatal life”.

NNL is considered to be the first one in a group of enamel microstructures, called accentuated striae of Retzius or Wilson bands, to be formed due to undefined stress factors disturbing enamel development [17]. Witzel et al [18] suggested that the severity of this kind of disturbance in amelogenesis is dependent of the intensity and duration of the stress factors. They introduced a theory that, under the influence of stress, secretory ameloblasts cross three levels of thresholds depending on the strength of the stress factor: 1) in case of a weak stress factor, the disturbance in enamel represents reduced secretory activity, but still formed prismatic enamel with shorter incremental spacing, 2) under stronger stress, aprismatic enamel is formed and 3) under strongest stress, disturbance in amelogenesis is severe and secretion of ameloblasts totally or temporarily ceases. Thus, according to our results, the prolonged delivery might be a stress factor strong enough to cause ameloblasts surpass the third threshold and transiently prevent ameloblast

secretion and result in a disturbance seen as narrower NNL. According to Witzel et al [18], an external intervention (suction cup or forceps) could act as an intensifying factor strengthening the stress effect on secretory ameloblasts. On the other hand, under weak stress factor, ameloblasts surpassing the first or second threshold, prism path can be detected and even daily cross striations are seen. It can be speculated that in short durations of delivery, the wide NLL (under light microscope) expresses the birth event and its aftermath, because the secretion is ongoing with only minor changes in the microstructure.

Eli and co-workers [9] reported a significant association between NNL width and the mode of delivery in normal children of undefined population (n = 147) with “normal” defined as with no history of systemic disorders related to pregnancy or labour and birth weight not less than 2500g. They reported that elective cesarean sections (n = 5) represented the narrowest lines, and assisted vaginal (n = 17, breech, forceps or vacuum delivery,) the widest, whereas the widths of NNL in spontaneous vaginal deliveries (n = 125) were inbetween. Our results are controversial to their data. Neither could Zanolli et al [10], on the basis of an undefined retrospective sample of 100 Italian school children, reproduce the results of Eli el al. Zanolli et al [10] observed the thinnest mean in NNL width in assisted vaginal deliveries (n = 5). Thus, their finding could argue for our speculative theory of transient ceasing of secretion of ameloblasts during stress caused by assisted vaginal delivery.

The present study includes some potential limitations. First, the sample was small. It was, however, based on an unselected population and randomized sampling and was thus representative of the deliveries in the study area. Another potential limitation is the use of several tooth types for analysis. Mahoney [19] suggested that the studies of accentuated striae in deciduous dentition should be made within the same tooth type groups, because “at any one time during enamel formation, each deciduous tooth type will have a different secretion and extension rate”, indicating poorly comparable measurements between tooth types. Results of Canturk et al. [11], using only second mandibular incisors (n = 24), observed a significant difference in NNL width between cesarean section and spontaneous vaginal birth mode, the line widening accordingly. Kurek et al [12] found significant differences in NNL width between central and lateral incisors, but could not show significant differences in width between cesarean section and spontaneous vaginal deliveries within one tooth type.

5. Conclusions

Neonatal line is demonstrable in virtually all deciduous teeth. Its width seems to be inversely proportional to the duration of delivery. Causes are largely unknown, but are speculated to result from altered amelogenesis induced by prolonged and intensified delivery-associated stress. Further research, including electron microscopic studies, is needed to understand the underlying mechanisms. Such research is underway.

References

[1] Ham A.W., Cormack D.H. Histology. USA: J.B. Lippincott Company; 1979

[2] Schour, I. The Neonatal Line in the Enamel and Dentin of the Human Deciduous Teeth and First Permanent Molar. J Am Dent Assoc. 1936; 23(10):1946–1955. doi:10.14219/jada.archive.1936.0277

[3] AlQahtani, S. J., Hector, M. P., & Liversidge, H. M. Brief communication: The London atlas of human tooth development and eruption. Amer J Phys Anthropol. 2010; 142(3):481–490. doi:10.1002/ajpa.21258

[4] Skinner M., Dupras T. Variation in birth timing and location of the neonatal line in human enamel. J Forensic Sci.1993; 38(6): 1383-1390

[5] Weber D.F., Eisenmann D.R. Microscopy of the neonatal line in developing human enaml. Am J Anat. 1971; 132: 375-392

[6] Sabel, N., Johansson, C., Kuhnisch, J., Robertson, A., Steiniger, F., Norén, J.G., Klingberg, G., Nietzsche, S. Neonatal lines in the enamel of primary teeth—A morphological and scanning electron microscopic investigation. Arch. Oral Biol. 2008; 53 954–963. doi:10.1016/j.archoralbio.2008.05.003

[7] Birch, W., & Dean, M. C. A method of calculating human deciduous crown formation times and of estimating the chronological ages of stressful events occurring during deciduous enamel formation. J Forensic Leg Med. 2014; 22:127–44. doi:10.1016/j.jflm.2013.12.002

[8] Birch W., Dean C. Rates of enamel formation in human deciduous teeth. Front Oral Biol. 2009; 13:116-120

[9] Eli I., Sarnat H., Talmi E. Effect of the birth process on the neonatal line in primary tooth enamel. J. Pediatr. Dent. 1989; 11: 220-223

[10] Zanolli C., Bondioli L., Manni F., Rossi P., Macchiarelli R. Gestation length, mode of delivery and neonatal line thickness variation. Hum Biol. 2011; 83(6): 695-713

[11] Canturk N., Atsu S., Aka P., Dagalp R. Neonatal line of fetus and infant teeth. An indicator of live birth and mode of delivery. Early Hum. 2014; 90:393-397

[12] Kurek, M., Żądzińska, E., Sitek, A., Borowska-Strugińska, B., Rosset, I., & Lorkiewicz, W. (2015). Prenatal factors associated with the neonatal line thickness in human deciduous incisors. HOMO - J Comp Hum Biol. 2015; 66(3):251–263. doi:10.1016/j.jchb.2014.11.001

[13] Kurek, M., Żądzińska, E., Sitek, A., Borowska-Strugińska, B., Rosset, I., & Lorkiewicz, W. Neonatal line width in deciduous incisors from Neolithic, mediaeval and modern skeletal samples from north-central Poland. Ann Anatomy. 2016; 203:12-18. doi:10.1016/j.aanat.2015.02.006

[14] Rautava P., Sillanpää M. The Finnish family competence study: Knowledge of childbirth of nulliparae seen at maternity health care clinics. J Epidemiol Community Health. 1989; 43:253-260

[15] Central Statistical Office of Finland. Handbooks / Statistics Finland, 17 (in Finnish, English summary) Classification of Socio-economic Groups. Helsinki, Finland: Statistics Finland, 1989

[16] Hillson S. Tooth development in human evolution and bioarchaeology. Cambridge, England: Cambridge University Press; 2014

[17] FitzGerald, C. M., & Saunders, S. R. Test of histological methods of determining chronology of accentuated striae in deciduous teeth. Amer J Phys Anthropol. 2005; 127(3):277–90. doi:10.1002/ajpa.10442

[18] Witzel C., Kierdorf U., Schultz M., Kierdorf H. Insights from the inside: histological analysis of abnormal enamel microstructure associated with hypoplastic enamel defects in human teeth. Amer J Phys Antropol. 2008; 136:400-414

[19] Mahoney P. Dental fast track: prenatal enamel growth, incisor eruption, and weaning in human infants. Amer J Phys Anthropol. 2015; 156:407-421

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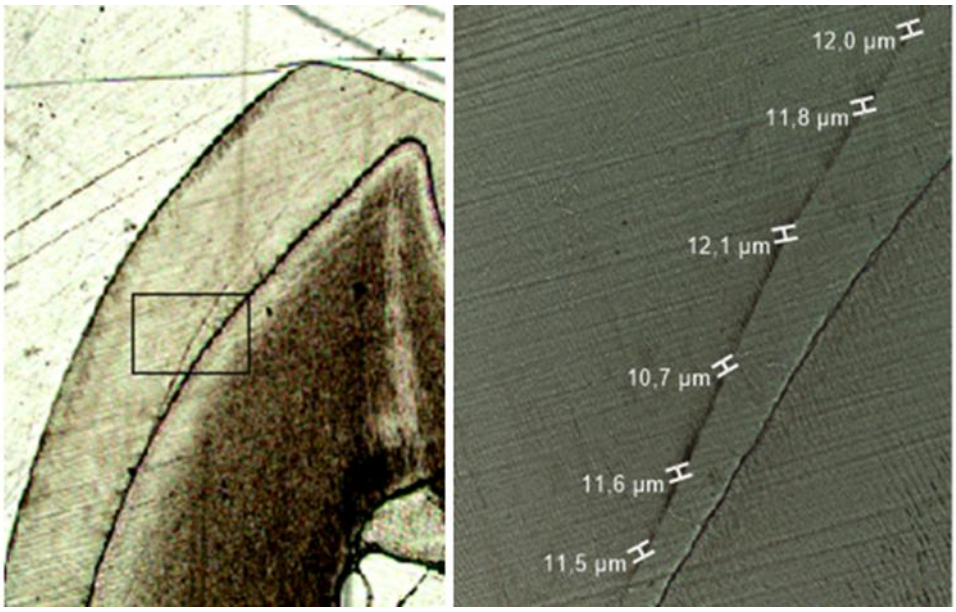

Fig. 1. Left) Maxillary right canine, light microscope image, Leica DM600B with 1x Leica DMC2900 camera, objective 1, 25x/0.04. Right) Square magnification with 10x/0.04 with six measurements of neonatal line width.

Table 1

A population study sample of loosened or removed deciduous teeth in children by sex, tooth type, and wear. Group A: teeth with no to moderate wear, Group B: heavy wear through enamel up to dentin

	Group A n = 54				Group B n = 75				All samples n = 129
	Boy	Girl			Boy	Girl			Boy+Girl
Sex	21	33			36	39			57+72
Tooth	Incisive	Canine	Molar	Total	Incisive	Canine	Molar	Total	Total
Maxillary	2	2	12	16	2	8	22	32	48
Mandibular	1	18	19	38	7	34	2	43	81
Wear									
None	1	8	21	30	-	1	9	10	40
In enamel	-	6	8	14	-	2	9	11	25
In enamel and dentin	2	6	2	10	9	39	6	54	64

Table 2

Neonatal line widths in teeth with no to moderate wear (Group A) and in those with heavy wear through enamel up to dentin (Group B).

NNL width (µm)	n	Min	Median	Max	Mean	SD
Group A	54	4.30	11.48	21.10	11.41	3.73
Group B	75	3.16	8.29	27.58	9.53	4.87
All	129	3.16	9.63	27.58	10.34	4.52

Table 3

Variation in duration of delivery and neonatal line width of vaginal deliveries (Group A) in a Finnish population sample

	n	Min	Max	Mean	SD
All Vaginal deliveries:	39				
Duration of delivery (min)		156	1688	536	263
NNL width (µm)		4.53	21.10	11.75	3.88
NNL width (log)		1.52	3.05	2.41	0.36
Spontaneous vaginal deliveries:	28				
Duration of delivery (min)		156	1688	559	282
NNL width (µm)		6.01	17.93	11.09	3.02
Assisted vaginal deliveries:	11				
Duration of delivery (min)		170	842	479	211
NNL width (µm)		4.56	21.10	13.43	5.32

Vaginal deliveries: NNL width vs. duration of delivery, $r = -0.41$, $p = 0.0097$; spontaneous vaginal deliveries: NNL width vs. duration of delivery spontaneous, $r = -0.33$, $p = 0.087$; assisted vaginal deliveries: NNL width vs. duration of delivery assisted, $r = -0.63$, $p = 0.040$

Table 4

Neonatal line width and mode of delivery in 54 (Group A) deciduous teeth

Mode of Delivery	n	Min	Median	Max	Mean	SD
Caesarean section	15	4.30	11.62	16.00	10.53	3.26
Spontaneous vaginal	28	6.01	10.83	17.93	11.09	3.02
Assisted vaginal	11	4.56	15.20	21.10	13.43	5.32

NNL width differences according to mode of delivery, p = 0.36