Forensic Science International 271 (2017) 87–91

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

# Deciduous neonatal line: Width is associated with duration of delivery

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ARTICLE INFO

*Article history:*
Received 27 May 2016
Received in revised form 2 December 2016
Accepted 11 December 2016
Available online 21 December 2016

*Keywords:*
Neonatal line
Forensic odontology
Long-term study
Mode of delivery
Duration of delivery
Population sample

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.



## 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 and 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

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http://dx.doi.org/10.1016/j.forsciint.2016.12.016
0379-0738/

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 and Eisenmann [5] to result from oblique cutting. The width of NNL is reportedly from few up to 30 μm [5,9,10,11–13]. 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 [9–11]. 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 socioeconomic 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.