ORIGINAL ARTICLE

Neonatal line as a linear evidence of live birth: Estimation of postnatal survival of a new born from primary tooth germs

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Abstract

Background: The presence of neonatal line indicates live birth and it is possible to estimate the exact period of survival of the infant in days by measuring the amount of postnatal hard tissue formation, and thus can be an evidence to the brutal act of infanticide. **Materials and Methods:** Primary tooth germs of both the arches were removed from the sockets of an infant who died few days after birth. Ground sections were made with hard tissue microtome. Decalcified sections were made from the crown of primary right mandibular canine and the sections were stained with hematoxylin and eosin. To visualize the neonatal line, the sections were subjected to light mocroscopy, polarized microscopy and scanning electron microscopy. A developing permanent molar from a one and a half year old boy and ten fully developed deciduous molars were used as controls. **Results:** The ground sections of all the developing tooth germs showed the presence of neonatal line and the analysis of enamel showed six distinct cross striations along the enamel rod length indicating the period of survival of the baby to be six days which was later confirmed with the hospital records. **Conclusion:** Neonatal line could be used as an evidence of infanticide. Accurate detection of neonatal line with advanced techniques could rewrite this supplementary evidence of infanticide into substantial evidence.

Key words: Infanticide, live birth, postnatal survival, neonatal line

Introduction

The brutal act of neonaticide, especially targeted against newborn female babies is a common practice in India. Most cases of neonaticide are not known to the outside world, and those cases which are brought before the law remain unproven due to lack of proper evidences. Forensic examination of the skeletonized remains belonging to the infants in the perinatal period is an enduring challenge in forensic medicine. The prime objective of forensic investigation in infanticide is to provide evidence against the claim of stillbirth. Distinguishing live birth from stillbirth would be a sturdy evidence to prove a case of "infanticide". If the soft tissue remains are intact, the changes occurring in the lungs, heart and the surrounding vessels at birth may provide valuable information regarding the independent existence of the child.[1] But, most often in forensic practice, by the time the body is retrieved, the soft tissue remains are decomposed and the body is putrefied and skeletonized. Though reasonable estimation of the period of gestation is possible using various skeletal parameters, these methods are not accurate and none of these methods help in distinguishing a live birth from a still birth.[2] In such a situation, the examination of developing tooth may provide a reliable answer pertaining to the fetal age, the possibility of a separate existence, and even the period of survival after birth.

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One such situation has been experimentally reviewed here, wherein, a linear secret code - the neonatal line, a hypomineralized line seen in the enamel of the developing tooth germ embedded deep in the jaws of an infant, serves as a supplementary evidence in separating infanticide from stillbirth. The neonatal line permits registration between developmental time and chronological age; subsequent formation time can be added for estimation of chronological age.[3] In a developing tooth, it is possible to use this information and the principles of incremental deposition of enamel to determine the infant's age at death. Since the neonatal line forms a clear limitation between the extent of prenatal and postnatal crown formation, it is independent of gestational age or size at birth.

Study background

Body of a full term baby who died six days after birth in our hospital due to respiratory distress was donated to the Department of Anatomy. The alveolar segments from the baby were retrieved. The ground sections obtained from the developing tooth germs were subjected to microscopic study to assess the postnatal survival of the baby.

Aim of the study

To demonstrate the presence of neonatal line in the developing primary tooth germs of a new born baby who died in the perinatal period using light, polarized and scanning electron microscope (SEM).

Objectives of the study

- To use the presence of neonatal line as an evidence of live birth against stillbirth; and
- To estimate the period of survival of the infant using neonatal line as the guideline.

Materials and Methods

The study sample consisted of primary tooth germs retrieved from the jaws of an infant who died few days after birth. The alveolar segments of the baby were retrieved [Figure 1]. After locating the position of tooth germs from the occlusal radiograph of the alveolar segments [Figure 2], the developing tooth germs of primary dentition [Figures 3-5] from both the arches were removed from the socket. Decalcified sections were made from the crown of primary right mandibular canine and the sections were stained with hematoxylin and eosin. For preparing ground sections, the calcified part of the developing tooth germs were separated out and stored in 70% ethanol. Teeth were oriented and embedded in epoxy resin and ground sections of 150 μ thickness were made longitudinally in a mesio-distal direction with hard tissue microtome. The sections obtained were placed in sodium hypochlorite solution for ten minutes, dehydrated using increasing grades of alcohol, and mounted on glass slides. The slides were subjected to microscopic study using light and polarized microscopy to visualize the neonatal line.

For SEM study, sections from each tooth were etched in 30% phosphoric acid for 30 seconds and carefully rinsed with de-ionized water and dehydrated in the air. The sections were mounted on sample holders for SEM and sputter coated with gold, the thickness of the coating was 15-20 nm. They were then examined in SEM (Joel-JSM 6490LA) for neonatal line.

One developing permanent molar from a one and a half year old boy whose tooth was removed along with the associated dentigerous cyst and ten fully developed deciduous molars were used as controls.

Observations

The ground sections of all the developing tooth germs obtained from the body of the new born baby showed the presence of neonatal line. Under light microscope, the neonatal line was seen as a distinct dark line, closer to the outer surface of the enamel and the line was found to be running parallel to the outer surface. In sections of incisors, the line was continuous and straight [Figure 6], whereas in canines and molars which are far less mineralized, the line was indistinct and appeared to be scalloped [Figure 7]. In the decalcified sections from the right mandibular canine, the enamel was retained and along the serrated outer mineralizing front of the enamel a layer of ameloblasts was noticed [Figure 8]. But neonatal line could not be identified in the decalcified sections.

The neonatal line could be better visualized using polarized microscope, where the line appeared as a distinct positive birefringent band [Figure 9].

In SEM, the neonatal line appeared as an indistinct white line which was scalloped. The path of enamel rods could not be traced as the surface was covered with a structure-less film [Figure 10].

The neonatal line could also be demonstrated in the ground sections of all the fully developed deciduous molar teeth and the developing permanent first molar, which were used as controls [Figure 11a and 11b]. Under SEM, the neonatal line was seen as a prominent white line, which was straight and did not show any scalloping [Figure 11c].

The presence of neonatal line and the considerable amount of enamel formed beyond it in the developing tooth germs of the newborn infant demonstrated by light microscope, polarized microscope, and SEM indicated that the baby was born alive and had survived for some days before death. In both maxillary and mandibular incisors, the cross striations on the enamel rod that represents the daily incremental deposition of enamel were clearly visible under both light and polarized microscope. Between the neonatal line and the outer

Figure 1: Mandibular alveolar segment retrieved from the baby

Figure 2: Occlusal radiograph of the mandibular alveolar segment from the baby showing developing tooth germs

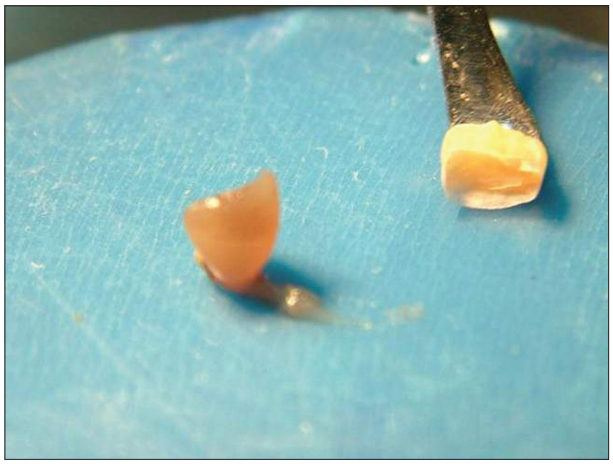

Figure 3: Developing deciduous maxillary central incisor with the mineralized crown separated out

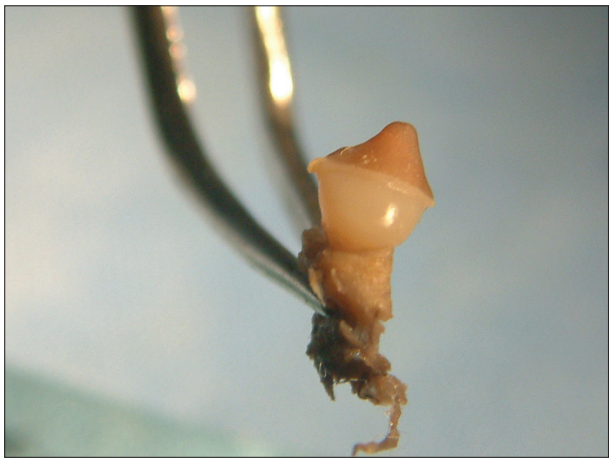

Figure 4: Developing deciduous mandibular canine showing calcification in the cuspal third

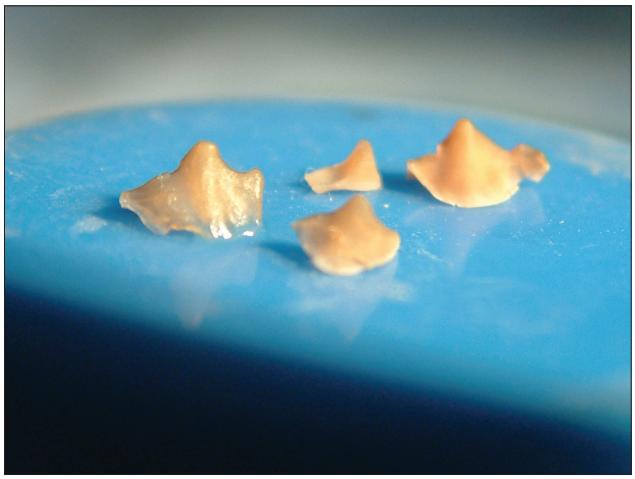

Figure 5: Calcified crowns of developing molar teeth

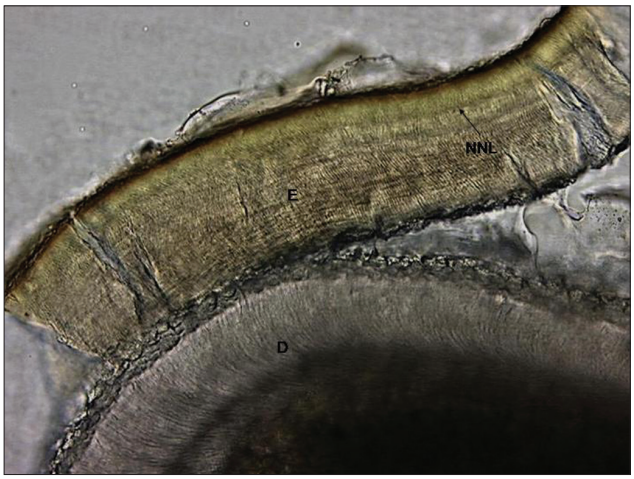

Figure 6: Light microscopic view of ground section of retrieved mandibular central incisor tooth germ exhibiting neonatal line (40×) (NNL-Neonatal line; E-Enamel; and D-Dentin)

surface of the enamel which represents the time of death, six distinct cross striations could be counted along the enamel rod length [Figure 12 a and 12 b] indicating the period of survival of the baby to be 6 days which was later confirmed with the hospital records.

Discussion

Discrimination between still birth and live birth is important both for forensic cases and for paleoepidemiological studies.

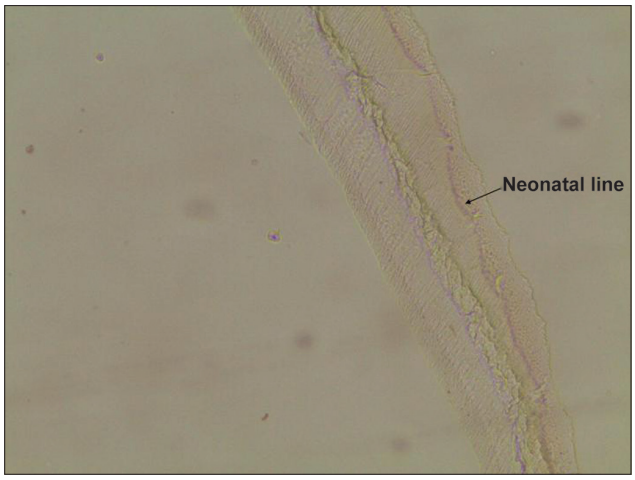

Figure 7: Ground section of mandibular canine crown showing neonatal line. Note the scalloping of the neonatal line

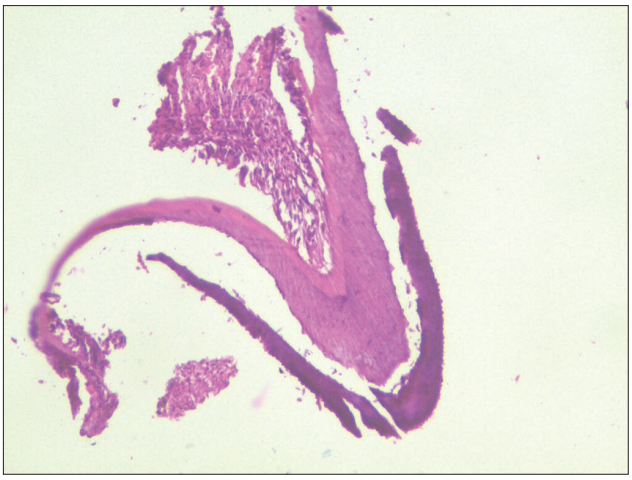

Figure 8: Decalcified section of mandibular canine showing scalloped outer margin of enamel. Note the ameloblasts lodged in the concavities of the enamel surface (H and E, 10×)

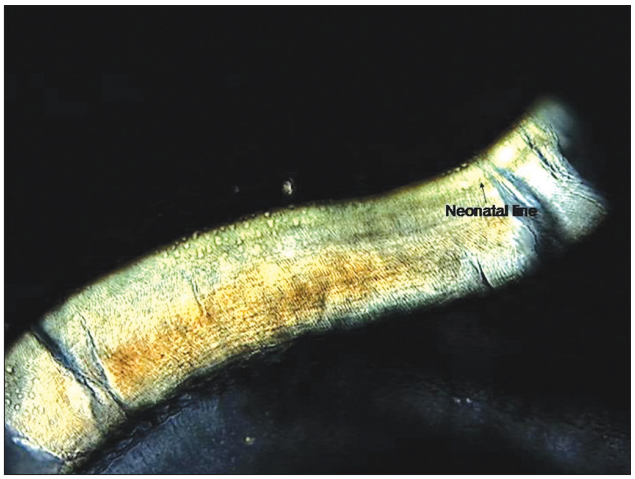

Figure 9: The polarized microscopic view of the ground section shown in Figure 6 showing neonatal line

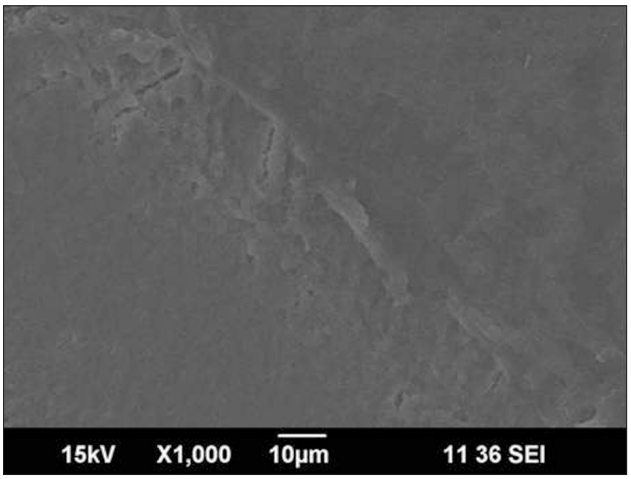

Figure 10: Scanning electron microscopic view of maxillary central incisor showing neonatal line

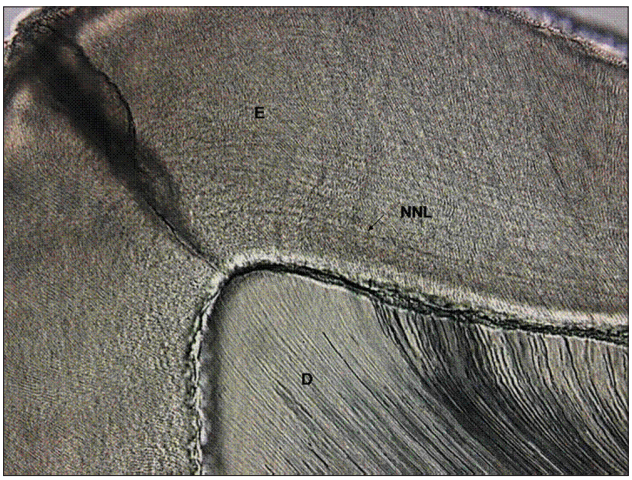

Figure 11a: Neonatal line in the ground section of developing mandibular first molar from a one and a half year old boy (light microscopy)

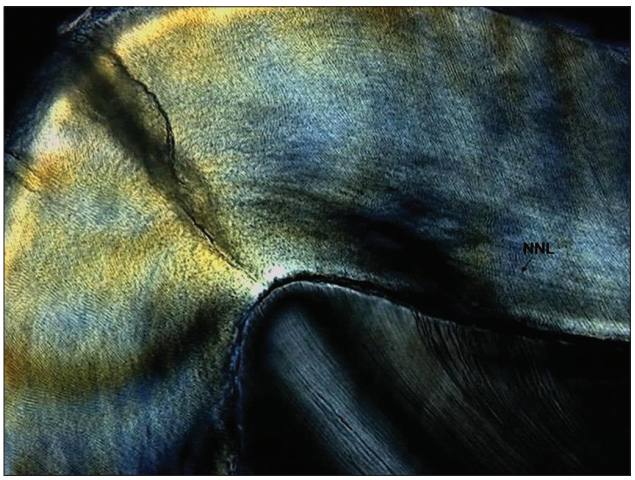

Figure 11b: Polarized microscopic view

Dental development data are usually based on formative or development changes and are good predictors of age from in-utero until about early twenties.[4] Dental assessment gives more precise age information than all anthropometric

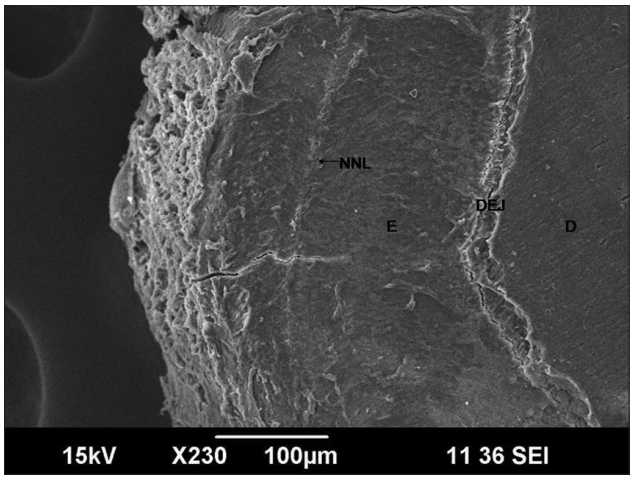

Figure 11c: Scanning electron microscopic view (NNL-neonatal line; E-enamel; D-dentin; and DEJ-dentino enamel junction)

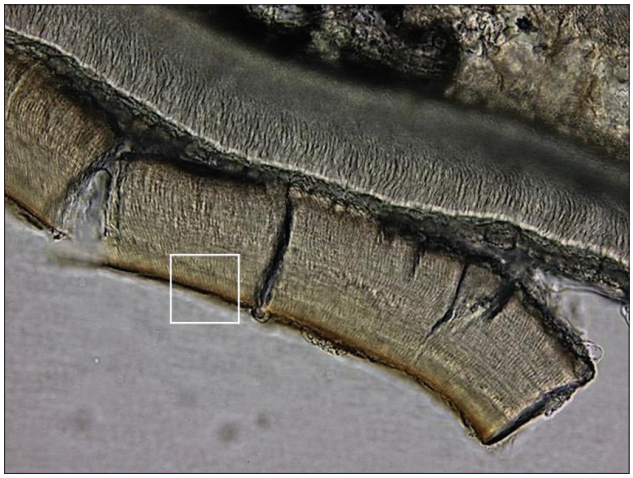

Figure 12a: Six cross striations across the enamel rod in the post natal enamel of mandibular central incisors indicating that the baby survived for a period of 6 days after birth

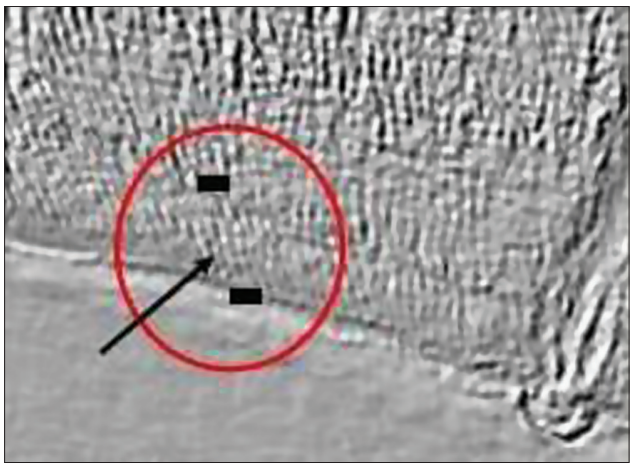

Figure 12b: The selected area in Figure 12a magnified

measurements.[5] In children, emergence status of deciduous teeth had been used to estimate the age at time of death in forensic investigations.[6] But, not many studies using dental criteria exist when it comes to the estimation of age of infants in the perinatal period. A study based on the metric tooth development of central incisors had shown that the age of the fetus and infants can be determined by the measurement of a single central incisor.[7] Since variation in the size of the tooth occur between individuals, age estimation based on dimensions of developing teeth seems to be less reliable.

The mineralization of dental hard tissues like enamel, dentin, and cementum follows a regular incremental pattern. At birth, deciduous tooth germs are incomplete and the enamel is only partially mineralized. Physiological upset in the cellular activity of ameloblasts at birth results in the formation of an accentuated incremental line known as neonatal line. Neonatal line is an optical phenomenon produced due to alteration in the dimension, direction, and degree of mineralization of the enamel prisms[8] caused by the biological stress imposed by a sudden change from intrauterine to extra uterine life. Neonatal line represents the first enamel formed after birth and it separates prenatal enamel from postnatal enamel. A similar hypomineraralized line can be seen in dentin also, but the mineralization pattern and the non-reparative nature of enamel make the neonatal line in enamel more distinct and reliable than the one in dentin. Since the deciduous tooth germs and the first permanent molar begin calcifying by the fifth lunar month (20th week in utero), and the mineralization continues for some period of time after birth, all these teeth posses neonatal line. As it forms a precise line of demarcation between prenatal and post natal enamel indicating the time of birth, the presence of neonatal line in a developing tooth indicates a live birth and a separate existence.

The neonatal line was identified in the enamel of all the developing tooth germs which were subjected to the microscopic study indicating the baby was born alive and it had a separate existence. In our study, we found that the neonatal line and the cross striations are visualized better in incisors when compared to canines and molars, and hence, the primary incisor must be the "tooth of choice" for the estimation of post natal survival of new born babies. The scalloped pattern of neonatal line seen in canines and molars under both light and polarized microscope may be explained in terms of the shape of the terminal secretary end piece of the ameloblasts. Similar scalloping can be noticed on the superficial surface of the enamel in the decalcified section where the ameloblasts can be seen lodged within the concave margins [Figure 4]. In the ground sections of incisors, the neonatal line that appeared as a straight line under light microscope showed scalloping under SEM. But in the developing first molar from a one and a half year old boy and in the fully developed deciduous molars which were used as controls, the neonatal line was rather straight. Based on these observations, it was understood that as mineralization progresses, neonatal line which initially appears as a scalloped line, straightens out and

becomes distinct as more and more layers of enamel gets deposited over it.

The estimation of period of survival of an infant in the perinatal period using neonatal line as the line of reference seems to be more accurate as it gives the exact age of the baby in days. The exact timing of enamel formation is always the same for a given individual, though it shows slight variation between individuals and species.[9] In primary dentition, the rate of enamel formation varied between 2.5-4.5 μ/day.[10] Cross striations seen across the enamel rod represents daily incremental deposition of enamel,[11] and hence, the cross striation count along the prism length between the neonatal line (birth line) and the last layer of enamel formed (death line) will give the exact age of the baby in days. In the present study, the six days of post natal survival estimated from the cross striation count was found to be accurate as the chronological age of the baby was available for comparison.

The main limitation of using neonatal line for the assessment of postnatal survival of infants is that most of the infanticides occur immediately after birth, but a couple of days of survival are necessary before the neonatal lines could be detected. As per the evidence from contemporary literature, neonatal lines can be demonstrated with light microscope only if considerable thickness of post natal enamel deposition takes place. In practice, that means the baby should survive for a period of around 3 weeks for the neonatal line to be visible.[1] But, in this study, we could demonstrate neonatal line using light microscope in a baby who survived for just 6 days. The detection of the neonatal line depends on various factors like the axis of the tooth section, section thickness, and the light source used and also, the absence of this line is not an indication of still birth.[12] So, it can be assumed that in the previous studies, procedural errors would have resulted in the absence of the neonatal line in infants who survived for a period less than 3 weeks. According to Whittaker and MacDonald,[1] it should be possible to distinguish postnatal enamel from prenatal enamel within a day or two after birth under SEM. Though SEM examination of the ground sections of tooth germs in our study also showed the presence of neonatal line, the period of survival of the baby could not be determined using SEM, as the enamel rods were not clearly visible.

Conclusion

Neonatal line and its significance in establishing the period of separate existence could be used as a proof, if required, in substantiating the criminal nature of infanticide. More accurate and earliest detection of this line particularly within hours after death by means of advanced techniques could rewrite this supplementary evidence of possible infanticide into substantial evidence and more studies need to be done in this direction.

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