WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 13, Issue 2, 753-765. Research Article ISSN 2277–7105

COMPARATIVE EVALUATION OF HISTO-MORPHOLOGICAL ASPECTS AND CHEMICAL COMPOSITION OF DENTAL HARD TISSUES IN PRIMARY TEETH FROM PRETERM AND FULL-TERM CHILDREN- AN IN VITRO STUDY

Dr. Sabin Sathyan*, Dr. Poornima P., Dr. Mallikarjuna K., Dr. Nagaveni N. B., Dr. Roopa K. B., Dr. Praveen Kumar Bali

3426, Sri Manjunatha Nilayam 10th Cross Kumaraswamy Layout II Stage, Bangalore, Karnataka, India.

Article Received on
28 November 2023,

Revised on 18 Dec. 2023,
Accepted on 08 Jan. 2024

DOI: 10.20959/wjpr20242-30987

***Corresponding Author**
Dr. Sabin Sathyan
3426, Sri Manjunatha Nilayam 10th Cross Kumaraswamy Layout II Stage, Bangalore, Karnataka, India.

ABSTRACT

Background: Children born prematurely suffer from various systemic conditions. These complications have been reported to cause disturbed mineralization in primary teeth. **Aim:** To evaluate and compare the enamel histomorphology and chemical composition of primary teeth in preterm and full-term children. **Design:** This study was an in vitro comparative study of 6 months duration. A total of 20 primary teeth from children born full-term (Group A) and 20 primary teeth from children born preterm term (<37 weeks, Group B) were collected from Davangere district of Karnataka. They were examined for the histo-morphological aspects using Scanning Electron Microscope (SEM) and for the chemical composition by X-Ray microanalysis (XRMA). **Statistical analysis:** SPSS software version 25.0 was used. Non-parametric-Mann Whitney U test and unpaired t-test were used. **Results:** Histo-morphological analysis revealed that the enamel rods in preterm group were less distinct than that from the full-term group. Chemical analysis showed preterm group had significantly lower ($p<0.05$) calcium content compared to full-term group. Nitrogen and Sulfur content were found to be significantly higher ($p<0.05$) in teeth from preterm group. **Conclusion:** Histo-morphological and chemical analysis reveals that the primary teeth in children born preterm undergoes certain mineralization defects when compared to children born full-term.

KEYWORDS: Preterm; Full-term; Scanning electron microscope; X-Ray microanalysis; Primary teeth.

INTRODUCTION

It is a well-documented fact that children born preterm experience a variety of oral complications associated with their premature births.[1] Although the survival rate of children born prematurely has drastically increased, these children suffer from certain medical conditions such as perinatal asphyxia; hyperbilirubinemia; cardiovascular, respiratory, neurological and nutritional deficiencies; gastrointestinal problems; and/or infections. All of these conditions have been reported to be etiological factors behind the disturbed mineralization in primary teeth. There is an increase in the prevalence of mineralization disturbances seen in the primary teeth of children born prematurely, that is, < 37 gestational weeks.[2]

Dental developmental defects seen in children born prematurely can also be due to the deprivation of normal mineral stores in the body. It is also reported that the major accumulation of calcium and phosphorus takes place during the third trimester of pregnancy. Premature birth of a child therefore results in inadequate calcium and phosphate accumulation in the body. Other postnatal complications also play a role in the effect of disturbed calcium metabolism during the mineralization of teeth.[2]

Morphological studies done on sections of primary teeth from premature infants using polarised light and microradiography revealed a high frequency of hypo mineralized enamel. A scanning electron microscope can provide an ultra-structural view of the enamel. There are very few studies on the chemical composition in enamel and dentin of primary teeth from preterm children.[3]

Hence, this study was conducted to investigate the morphological appearance of primary enamel from preterm infants utilizing a scanning electron microscope and to analyse the content of certain elements in the enamel and dentin of primary teeth from preterm infants by X- Ray Microanalysis (XRMA). The results were then compared with the analyses of enamel and dentin in primary teeth from children born with full term.

METHODOLOGY

This study was an in vitro comparative study of 6 months duration conducted in the department of Pediatric and Preventive Dentistry, College of Dental Sciences, Davangare, Karnataka. A total of 20 exfoliated or extracted primary teeth from children born full-term (Group A) and 20 primary teeth from children born preterm (before gestational age of 37 weeks, Group B) with intact enamel and without known prenatal, neonatal and postnatal disorders were collected from the neonatal centre of Chigateri hospital in Davangere district of Karnataka. Records of preterm births were collected from the hospital and children who were nearing their age for natural exfoliation of primary teeth were selected. The families of these children were contacted and were asked to store the exfoliated teeth in saline and report to the department. Ethical clearance to conduct the study was obtained from the Institutional Ethical Review Board, College of Dental Sciences (CODS), Davangere, Karnataka. (Ref No. CODS/2066 /2020-2021).

Tooth preparation

The teeth collected were embedded in epoxy resin. One longitudinal serial section with a thickness of approximately 100µm from each sample was prepared using a hard tissue microtome for examining the morphological aspects using Scanning Electron Microscope (SEM) following which the chemical composition was examined by X-Ray microanalysis (XRMA). Teeth obtained for both the groups- preterm and full term were prepared the same way.

SEM Analysis

The prepared specimens were etched for 30 seconds 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.[3] The sections were studied under SEM to analyse and compare the orientation and overall appearance of the enamel prisms in primary teeth from preterm children and full-term children.

X-Ray Microanalysis

After SEM analysis, these samples were then mounted using carbon tape on sample holders for analyzing the chemical characteristics.[2] Assessment of main chemical constituents of hydroxyapatite and organic structure of enamel and dentin like Calcium (Ca), Phosphorous (P), Nitrogen(N) and Sulphur(S) were done by X-Ray microanalysis using the NIST-DTSA II (Desktop spectrum analyzer) software. The measurements were taken from enamel at three

levels- 10µm below the surface, one-half of the enamel thickness and 10µm above the Enamel-Dentin junction (EDJ). Similarly, measurements from dentin were also taken from three levels- 10µm below the EDJ, at one-half of the dentin thickness and 10µm above the pulp chamber.

Statistical Analysis

Statistical analysis was carried out using the SPSS software version 25.0.

The data obtained was statistically analysed using Mann Whitney U test in case of values that are non-normally distributed and student t test in case of values that are normally distributed. A p-value of less than 0.05 was considered to be statistically significant

RESULTS

SEM analysis

At 100x magnification, the enamel showed certain changes in the surface topography of the enamel in both the groups. A smooth enamel surface was seen in group A with no discontinuity in the enamel rod pattern whereas in group B it showed some discontinuity and a roughened surface. (Fig 1)

The SEM analysis also showed that the enamel from Group A (full-term) had enamel rods where the sections passed through the “heads” or “bodies” of one row of rods and the “tails” of an adjacent row.[4] This produced an appearance of rods separated by interrod substance (Fig 2). The rods were seen to follow a wavy course through the enamel structure. Images of Group A also showed regular and continuous enamel rods.

Group B (preterm group) had certain distinct differences as compared to the normal enamel seen in group A. A difference in the overall appearance of the prisms were noted in preterm enamel. Images of group B showed an irregular and haphazard pattern of rods which are discontinuous. A break in the continuity of enamel rods can be clearly observed in group B. (Fig 3). Also the diameter of enamel prisms were found to be lesser in preterm enamel and the width of interprismatic space was larger. (Fig 4)

X-Ray microanalysis

The mean enamel calcium levels and calcium: phosphorous ratio were significantly greater in group A (full-term) when compared to group B (preterm) 10µm under the surface and at one-half of the enamel thickness. The mean enamel phosphorous and sulfur levels were not

significantly different between the two groups at any level of the tooth. The mean enamel nitrogen levels were significantly greater in Group B (preterm) at all levels of the tooth.(Table 1)

The mean dentin calcium levels were not significantly different between the two groups at any level of the tooth. The mean dentin phosphorus levels were significantly greater in group B (preterm) - at one-half of dentin thickness and 10µm above the pulp chamber of the tooth. The mean dentin nitrogen and sulfur levels were significantly greater in group B (preterm) at all levels of the tooth. The mean dentin calcium: phosphorus ratio was significantly greater in group A (full-term) 10µm above the pulp chamber.(Table 2)

Tables

Table 1: Inter-group comparison of enamel calcium, phosphorus, nitrogen, sulphur and calcium: phosphorus ratios at three levels- DEJ, middle part and near pulp chamber.

Level	Group	Calcium	phosphorus	Nitrogen	sulphur	Ca/p ratio
10µm under the surface	Normal	36.03±3.12	16.61±1.01	0.95±0.13	0.044±0.015	2.17±0.25
	Preterm	34.42±2.47	16.73±0.63	1.29±0.27	0.044±0.012	2.05±0.11
	P value	0.027*	0.946	<0.001*	0.778	0.025*
Half of enamel thickness	Normal	34.81±2.75	16.50±0.90	1.04±0.20	0.047±0.015	2.11±0.22
	Preterm	33.51±1.46	16.57±0.75	1.32±0.27	0.042±0.008	2.02±0.14
	P value	0.006*	0.946	0.001*	0.310	0.045*
10µm above the DEJ	Normal	34.15±3.05	16.86±0.72	1.44±0.50	0.046±.013	2.02±0.18
	Preterm	33.47±1.78	16.45±0.67	1.86±0.38	0.041±0.014	2.03±0.13
	P value	0.102	0.101	0.001*	0.145	0.787

P value<0.05

***=Significant**

Table 2: Inter-group comparison of dentin calcium, phosphorus, nitrogen, sulphur and calcium: phosphorus ratios at three levels- DEJ, middle part and near pulp chamber.

Level	Group	Calcium	phosphorus	nitrogen	sulphur	Ca/p ratio
10µm under the EDJ	Normal	17.09±2.53	12.49±2.08	3.61±.69	0.33±0.08	1.40±0.311
	Preterm	18.55±3.92	13.12±1.46	4.28±0.74	0.43±0.11	1.42±0.313
	P value	0.172	0.272	0.006*	0.005*	0.842
Half of dentin thickness	Normal	15.40±2.78	10.36±1.54	2.80±0.51	0.28±0.06	1.53±0.452
	Preterm	16.11±3.26	11.93±1.15	3.63±0.54	0.34±0.09	1.36±0.320
	P value	.464	0.001*	<0.001*	0.010*	0.189
10µm above the pulp chamber	Normal	8.86±3.31	6.98±2.30	2.10±0.53	0.20±0.04	1.35±0.626
	Preterm	8.43±2.74	10.40±1.53	2.86±0.65	0.27±0.07	0.826±0.292
	P value	0.658	<0.001*	<0.001*	0.002*	0.001*

P value<0.05

***=Significant**

Figures

Figure Legends

Figure 1: a) Enamel surface of group A- Full-term (100x magnification)

b) Enamel surface of group B- Preterm (100x magnification)

Figure 2: a) Enamel pattern in group A- Full-term (3000x magnification)

b) Enamel pattern in group B- Preterm (3000x magnification)

Figure 3: a) Continous enamel rods in group A- Full-term (3000x magnification)

b) Discontinous enamel rods in group B- preterm (3000x magnification)

Figure 4: a) Distinct enamel rods with less interrod diameter

b) Less distinct enamel rods with increased interrod diameter

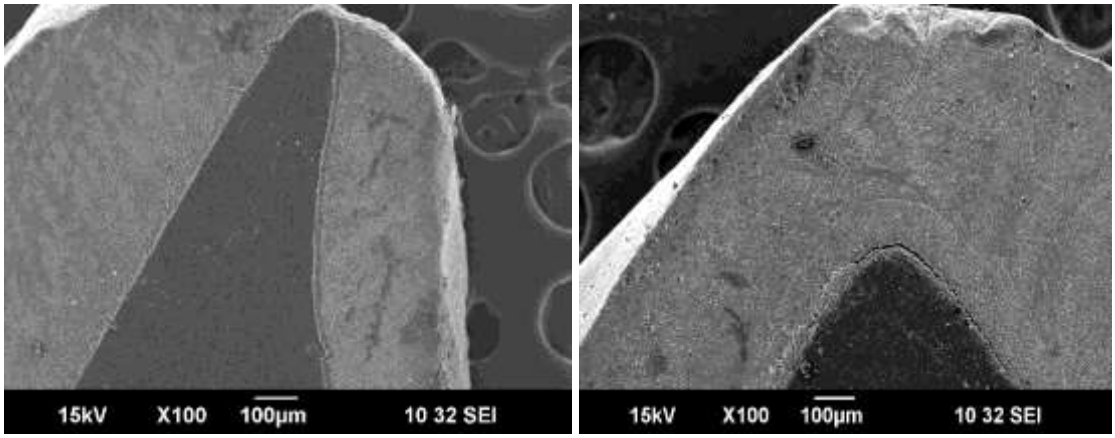

Figure 1.

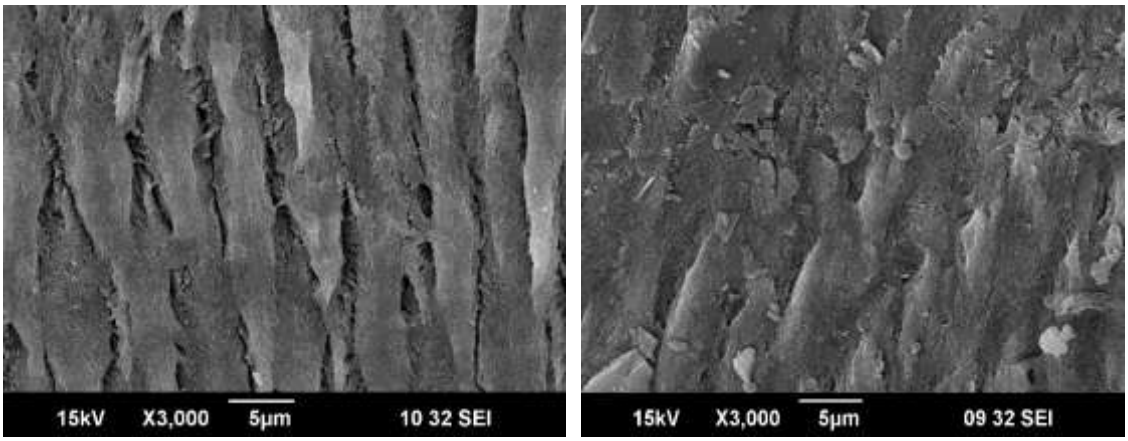

Figure 2.

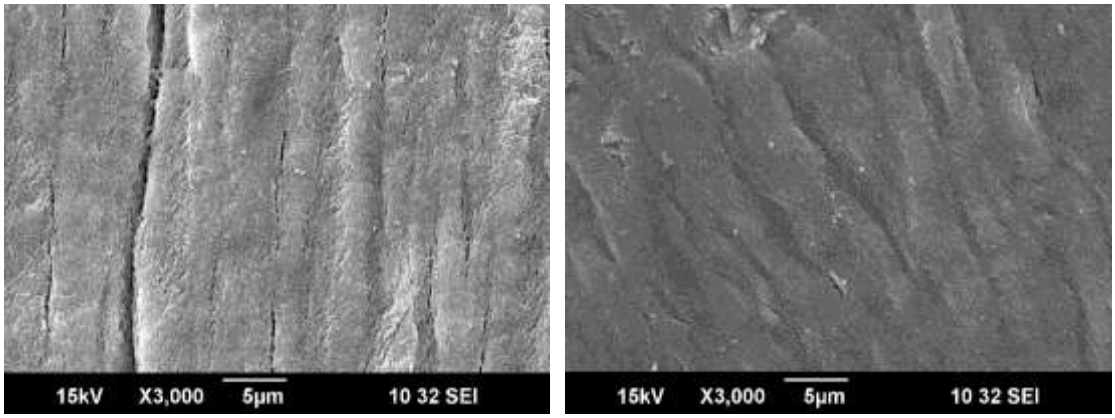

Figure 3.

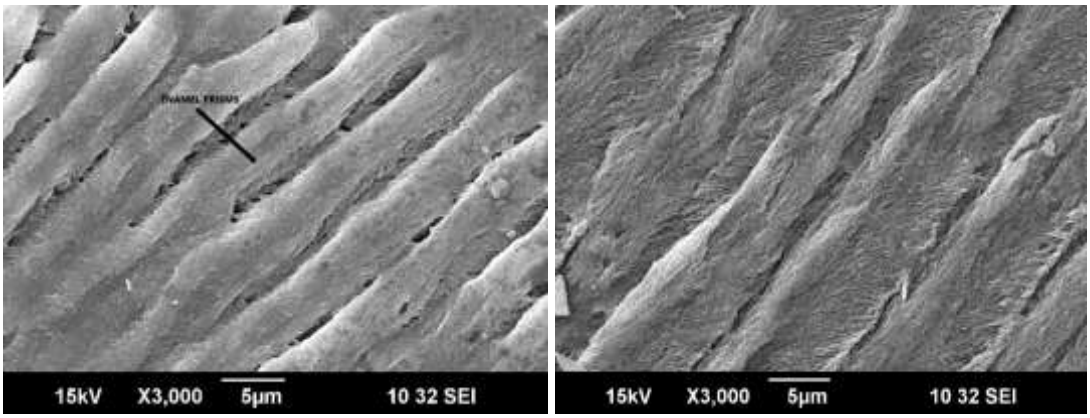

Figure 4.

DISCUSSION

The World Health Organization (WHO) defines premature or pre-term birth as a newborn born less than 37 weeks gestation or within 259 days of the last menstrual period.[2] Premature infant survival rates vary with birth weight, ranging from 98 percent (2000-2500 g) to 26 percent (750 g).[5]

Surviving babies may develop complications that are easily diagnosed and monitored, such as respiratory distress, neurological and cognitive issues, and the risk of developmental delay.[6] The disturbed calcium metabolism during the first days of life, as well as the fact that the majority of calcium and phosphate accumulation occurs during the last trimester of pregnancy, may be important factors behind enamel aberrations in primary teeth. Various postnatal complications can also be a reason for these enamel disturbances.[3]

Various morphological studies done on enamel sections (Noren J 1983, Seow WK 2005, Rythen M et.al, 2008, N Sabel, 2012) taken from children born preterm show a high

frequency of enamel hypomineralisation. Changes in the chemical composition of enamel and dentin in the teeth from preterm children can be the reason for changes in degree of porosity of the primary enamel seen in these studies.[2]

No previous studies have examined both the morphological aspects and chemical composition of the enamel from preterm group. This study was conducted to analyse the histo-morphological aspects using SEM under 100x and 3000x magnification and to measure the chemical constituents of enamel and dentin.

In this study, SEM images at 100x magnification showed changes in the surface topography of enamel from preterm group. Roughened surface with irregularities were seen when compared to the smooth surface of enamel from full-term group. As prematurely born infants have a high rate of enamel developmental defects, the enamel appears rough, granular, and poorly mineralized. They also have low calcium stores and disrupted calcium metabolism, with low-birth-weight children being the most severely affected. This could be because, in preterm children, the majority of the enamel is mineralized after birth and is thus susceptible to a variety of factors that could disrupt the mineralization.[7]

SEM images at 3000x magnification has shown that the histo-morphology of teeth from pre-term children displayed a variety of changes in the enamel as compared to the teeth from full-term children. Enamel prisms were not regular and were haphazardly arranged in the teeth of preterm children when compared to the regular and continuous enamel prisms of the full-term group. These results show that despite the compensatory growth of tissues during the postnatal period, the enamel quality is affected.[2] These results are strongly supported by the study done by W. Kim Seow and W.G. Young in 2005.[3]

Enamel seen under SEM showed that the enamel from pre-term group had a less distinct structure when compared to that of the enamel from full-term group. This is in accordance with the study by Rythen M and Noren J (2008), and Klingberg G (2005). Various reasons like hypocalcemia, hypoxia and intubation have been attributed to cause enamel defects.[8] Pre-term born infants are often intubated and this could be one of the reasons why enamel defects are seen widely in these children.[9] Grahnen et. al, (1974) said that as a whole, preterm delivery does not increase the risk of enamel defects, but complications such as asphyxia or hyperbilirubinemia may explain the problem.[10]

In this study the chemical composition was examined by X-ray Microanalysis (XRMA). The values obtained denoted that the Ca content in the enamel from preterm group was generally lower when compared to that in the full-term group. It reached a statistically significant lower value 10µm below the enamel surface and at one half of the enamel thickness. This suggest a lower mineral content in the enamel from preterm group and it could be because of the mineralization defects occurring with preterm births. This is in accordance with the study done by Rythen N and Sabel N (2010). The values of enamel from full-term group obtained in this study is comparable with studies done by J Arends 1981 and W Dietz (2009).[2]

The values of P obtained from the enamel showed that there is no statistically significant difference between the preterm and full-term group. The Ca: P ratio was found to be significantly lower in the preterm group compared to the full-term group at the surface and one half of the enamel thickness. Study by Rythen N (2010) have given values in accordance with this study. Contradicting values have also been obtained in certain previous studies done by Lakomaa E.L (1977) and Keinan D (2006). Lower Ca: P ratio in preterm group can be because of the reduced inorganic content due to mineralization defects as confirmed in the histo-morphological analysis in this study.[3]

Organic matter of primary teeth from preterm and full-term children contains Carbon, Nitrogen, Sulfur, Oxygen, etc. In this study, N and S contents were analyzed in order to evaluate the organic matter in primary teeth from preterm and full-term children. N content in enamel and dentin from preterm group was found to be significantly higher in the preterm group when compared with the full-term group. It signifies the increased organic matter in the tooth. This result is in agreement with the study done by Rythen N (2008) where organic content was found to be greater in teeth from preterm children. Bohic S (1998) using the higher value of carbonates found in his study hypothesized that increase in organic matter has been found in relation to small enamel crystallites, which may result in porous enamel. Adding the result of this study to that hypothesize gives it more strength.[3]

The present study is the first to analyze sulfur content in the teeth from preterm children. The values indicate no significant difference between the preterm and full-term group in both enamels. Whereas the S content in dentin showed a significant increase in the preterm group. This can be due to the increase in organic content in dentin of preterm children as mentioned before. S values obtained in a study done by Yarova P et. al, (2021) on teeth from full-term children is not in accordance with the values obtained in the present study.[11]

Dentin in the teeth from preterm children showed decreasing amounts of Ca and P from the EDJ towards the pulp chamber. Although no significant difference was found in the level of Ca between preterm and full-term group, there was a significant increase in the level of P in preterm group compared to the full-term group. The lower Ca: P ratio in dentin compared to enamel can be explained by the dentin's higher organic content, which contributes to higher P values and thus lower Ca: P values. The Ca: P values in the dentin from preterm children was significantly lower near the pulp chamber as compared to the full-term group. The differences found in values from dentin cannot be solely attributed to preterm birth. There are various factors which can affect the dentin constitution such as reparative dentin formation and dentin resorption (Tjaderhane 2011).[11]

Primary tooth mineralization begins around the 14th week of intrauterine life and is completed around 12 months postnatally after normal gestational age (37- 40 weeks). Therefore, prenatal children usually miss out a few weeks of developmental period for teeth during the third trimester (G.W Suckling 1989). The porous outer enamel in the preterm children's primary teeth may pose an increased risk for caries.[12] Results from this study confirm the increased risk of caries that the primary teeth from preterm children possess.

A study by Iram Zaidi et, al. (2015) showed that preterm children displayed a variety of changes in the enamel like less enamel thickness, increased roughness, pits, etc. on the surface. In the preterm children, the prenatally formed enamel is the most reduced—at a level of approximately 5 to 13 times the thickness of the enamel of full-term children, which directly reflects the shortened duration in the prenatal stage of enamel formation.[7] The same findings have been shown by Grahnen et. al. as they also found a decrease in enamel thickness in low-birth-weight infants when compared with full-term. The reduced enamel in preterm children is likely to have resulted from both cessation/reduction of ameloblastic activity and the decreased supply of mineral to the developing teeth.[13] The reduced Ca and P content in preterm enamel as seen in this study supports this theory.

Studies by Garne et, al. (1979), Fearne and Brook (1993) and Kim Seow et, al. (2005) also showed that enamel in preterm children is thinner compared to that of full-term children. Their results also showed that compared to children that were born at full term, preterm children have smaller dental measurements. The lowered dimensions are most likely the outcome of substantial metabolic functional disturbances brought on by their preterm births. They have reported that this reduction in the teeth size is due to a significant reduction in

overall enamel thickness.[1] On the contrary, a study done by V. Harila et, al. (2003) suggest short gestation does not reduce the deciduous tooth crown size. Variations in tooth crown size are influenced by maternal effects, nutritional factors, and developmental disturbances.[14]

The strength of this study is that it is the second of its kind to analyze the chemical composition of preterm teeth. Whereas, elements like nitrogen and sulfur that have been analyzed in this study have never been analyzed and quantified in the teeth from preterm children before. The values obtained and the comparison with the teeth from full-term children have given new information on the developmental defects seen in enamel and dentin of the teeth from preterm children.

Further studies in the future can be done using a polarized light microscope to analyse the incremental lines and enamel thickness in a more effective manner. Analyzing the amount of trace elements such as molybdenum, fluoride, vanadium, strontium, and lithium in the teeth can help to understand the caries risk in preterm children.

Bullet Points

1. Histomorphology of enamel from preterm group showed some enamel defects including irregularly oriented and a less distinct prism structure when compared with the full-term group.
2. Analysis of the chemical constituents showed that the teeth from full-term group had significantly higher ($p<0.05$) Ca content when compared to the preterm group. P content was higher in the teeth from preterm group although it was not statistically significant.
3. Organic content analysis revealed a significantly higher ($p<0.05$) N and S content in the primary teeth of preterm children compared to the full-term group.

Why this paper is important to pediatric dentists?

• Enamel in primary teeth from preterm children is of hypomineralised hence early preventive treatment procedures could be emphasized.

Conflict of interest

No conflict of interest by authors.

BIBLIOGRAPHY

1. Seow WK, Young WG, Tsang AK, Daley T. A study of primary dental enamel from preterm and full-term children using light and scanning electron microscopy. Pediatric dentistry, 2005 Sep 1; 27(5): 374-9.
2. Rythén M, Sabel N, Dietz W, Robertson A, Norén JG. Chemical aspects on dental hard tissues in primary teeth from preterm infants. European journal of oral sciences, 2010 Aug; 118(4): 389-95.
3. Rythén M, Norén JG, Sabel N, Steiniger F, Niklasson A, Hellström AN, Robertson A. Morphological aspects of dental hard tissues in primary teeth from preterm infants. International Journal of Paediatric Dentistry, 2008 Nov; 18(6): 397-406.
4. Nanci, A, editor. Ten Cate's Oral Histology: Development, Structure, and Function. 9th edition Elsevier Health Sciences, Amsterdam, 2016.
5. Seow WK. Oral complications of premature birth. Australian dental journal, 1986 Feb; 31(1): 23-9.
6. Cortines AA, Corrêa-Faria P, Paulsson L, Costa PS, Costa LR. Developmental defects of enamel in the deciduous incisors of infants born preterm: prospective cohort. Oral diseases, 2019 Mar; 25(2): 543-9.
7. Zaidi I, Thayath MN, Singh S, Sinha A. Preterm birth: a primary etiological factor for delayed oral growth and development. International journal of clinical pediatric dentistry, 2015 Sep; 8(3): 215.
8. Aine L, Backström MC, Mäki R, Kuusela AL, Koivisto AM, Ikonen RS, Mäki M. Enamel defects in primary and permanent teeth of children born prematurely. Journal of oral pathology & medicine, 2000 Sep; 29(8): 403-9.
9. Norén JG, Ranggård L, Klingberg G, Persson C, Nilsson K. Intubation and mineralization disturbances in the enamel of primary teeth. Acta Odontologica Scandinavica, 1993 Jan 1; 51(5): 271-5.
10. Grahnén h, Sjölin S, Stenström A. Mineralization defects of primary teeth in children born pre-term. European Journal of Oral Sciences, 1974 Oct; 82(5): 396-400.
11. Tjäderhane L, Carrilho MR, Breschi L, Tay FR, Pashley DH. Dentin basic structure and composition—an overview. Endodontic topics, 2009 Mar; 20(1): 3-29.
12. Feuser E, Moreira Teixeira N, Valdelice Cruz P, Baccin Bendo C, Abreu LG, Martins Paiva S, Penido MG, Castro Martins C. Developmental Enamel Defects and Dental Caries in the Primary Dentition of Preterm Children. Journal of Dentistry for Children, 2021 Jan 15; 88(1): 40-5.

13. Grahnen H, Larsson PG. Enamel defects in the deciduous dentition of prematurely born children. Odontol Revy, 1958 Sep; 9: 193-204.
14. Harila V, Heikkinen T, Alvesalo L. Deciduous tooth crown size in prematurely born children. Early human development, 2003 Dec 1; 75(1-2): 9-20.