

**Prenatal Versus Postnatal Enamel Of Deciduous Second Molars With Reference To  
Neonatal Line  
(Elemental And Scanning Electron Microscopic Study)**

Rabab Mubarak<sup>1</sup>, Dina M.M. El- Said M. Hassouna<sup>2</sup>



<sup>1</sup>Professor of Oral Biology- Faculty of Dentistry- Cairo University & Deraya University.

<sup>2</sup>Lecturer of Oral Biology- Faculty of Dentistry - Fayoum University.

**Abstract— Background:** The exact mineral content of deciduous 2<sup>nd</sup> molars (DSM) in prenatal, postnatal enamel and neonatal line is not fully determined. **Aim:** To determine the morphology and mineral content of prenatal, postnatal enamel and neonatal line. **Material and Method:** 5 human exfoliated DSM were collected, each tooth was divided into two halves so that 10 samples were obtained. Prenatal, postnatal enamel and neonatal line were examined. Samples were assessed morphologically by light microscope (LM), stereomicroscope and Scanning electron microscope (SEM). Moreover the samples were assessed for mineral analysis and all data were statistically analyzed. **Result and Conclusion:** In DSM, Morphological study revealed that, postnatal enamel of DSM was more translucent in color than prenatal enamel in stereomicroscope. The neonatal line had chalky white appearance in stereomicroscope. SEM morphometric analysis showed an increase in surface area of postnatal enamel rods whereas, their number showed a decrease when compared to prenatal enamel. Elemental study showed a significant increase in Calcium (Ca) and Ca/P ratio weight % and non-significant decrease in Phosphorous (P) weight % when prenatal compared to postnatal enamel and when postnatal enamel compared to neonatal line. C weight % of prenatal enamel decreased significantly when compared to postnatal enamel and decreased non-significantly when postnatal enamel was compared to neonatal line. Magnesium (Mg) and sodium (Na) weight % were absent in prenatal enamel. Neonatal line showed a significant increase in Na weight % than postnatal enamel. Postnatal enamel Mg weight % showed a non-significant increase when compared to neonatal line.

**KEYWORDS:** Prenatal enamel, postnatal enamel, Neonatal line, Ca/P ratio, EDXA.

**Introduction:**

Early loss of badly decayed DSM is a common problem in children which results in reduction of arch length. So, successor teeth become predisposed to crowding, rotation or even its impaction. Thus, maintaining DSM in its place and maintaining it from decay is one of the significant clinical goals to be achieved in dental clinics (Leite-Cavalcanti et al., 2008 and Ahamed et al., 2012). The cell which form enamel is called ameloblast, where enamel matrix is laid increment by increment resulting in incremental lines of Retzius. These lines are indices of ameloblast periods of activity and periods of rest. Maturation of enamel matrix starts upon laying down the 1<sup>st</sup> layer of enamel with 30% minerals which is called 1ry maturation of enamel. During maturation stage of ameloblast the proteins become reabsorbed and inorganic component are laid down to reach 96% maturation which is called 2ry maturation. Furthermore, 3ry maturation occurs after loss of ameloblast and eruption of tooth in oral cavity from minerals in saliva and food intake. The inorganic component of enamel is mainly hydroxyapatite crystals (Belikov et al., 2008; Alaluusua, 2010; Seow, 2014; Berkovitz et al., 2017; Zhang, 2018).

Enamel of all the deciduous teeth and first permanent molars develops partly before and partly after birth. Neonatal line separates enamel formed prenatally from enamel formed postnatally. Neonatal line is considered as a prolonged period of rest of ameloblast as it occurs due to abrupt change in the environment and nutrition of the newborn infant. The increased period of ameloblast rest in neonatal line of enamel makes it hypomineralized which makes it highly susceptible to caries. Carious process in enamel is a dynamic one with phases of demineralization alternating with phases of re-mineralization (Macchiarelli et al., 2006; Birch & Dean, 2009; Mishra et al., 2009; Nava et al., 2017; Dean et al., 2020; Dantas et al., 2020). However, the total amount of minerals and its relation to morphology of prenatal, postnatal enamel and neonatal line is not well understood which brought the aim of the present study. **Aim of the study:** To determine the quality of prenatal, postnatal enamel and neonatal line of DSM by elemental and morphological analysis.

### **Material and Methods:**

Five non-carious exfoliated human DSM, were collected from the out-patient clinics of Faculty of Oral and Dental Medicine, Cairo University after obtaining patient consent and acceptance from Research Ethics Committee of Faculty of Dentistry–Ain Shams University. The teeth were cleaned and kept hydrated in saline solution. Teeth were sectioned mesio-distally in a vertical direction into buccal and lingual halves using very thin diamond disc with low speed micro motor under water spray to avoid heat generation, so that each half has one sound enamel surface. Finally, Ten halves of sound enamel surface were obtained (n = 10), prenatal enamel; postnatal enamel and neonatal line were examined.

**Sample size calculated based on previous data** Martinović, et al., 2015, for Ca% with mean and standard deviation; 28.8, 1.03 for healthy enamel and 27.06 and 1.47 for hypomineralized enamel to achieve power 80% at a significant level 0.05m. Thus, **ten samples per group were needed.**

#### **1- Morphological examination:**

The ground sections of DSM with neonatal line were examined and photographed using Stereomicroscopy (Leica S 8Apo) as well as LM (Leica DM300) to reveal the morphological characteristics of the neonatal line at the Central Laboratories for Research & Mining, Egyptian General Authority for Mineral Resources - Ministry of Petroleum.

#### **2- Ultrastructure examination and morphometric analysis:**

The ground sections of DSM with neonatal lines were examined by scanning electron microscopy. The sections were etched with 37% wt phosphoric acid for 30 seconds and carefully rinsed with deionized water and dehydrated in air for 24 hours before final dehydration in an oven at 60°C. The dehydrated specimens were sputter coated with gold before SEM examination (Electronic Probe Microanalyser Jeol JAX- 840A). The scanning photomicrographs were morphometrically analysed regarding the number and diameter of enamel rods in both prenatal and postnatal enamel using Leica Owen 500 image analyzer computer system (Leica Imaging System Ltd., Cambridge, UK in Research unit in Faculty of Dentistry, Cairo University).

#### **3- Chemical composition examination:**

These specimens were used for the assessment of certain chemical composition using Energy Dispersive X-ray Analysis (EDX). X-ray detector system (EDX, INCA-X-sight) attached to a SEM (Jeol JAX- 840A) was used. Three readings were taken at the neonatal line, above the neonatal line (postnatal enamel) and below it (prenatal enamel).

#### **4- Statistical Analysis:**

All Data were collected, tabulated and subjected to statistical analysis. Statistical analysis is performed by SPSS in general (version 20), while Microsoft office Excel is used for data handling and graphical presentation. All variables are described by the Mean, Standard Deviation (SD), the Range (Minimum – Maximum), Standard Error (SE) and 95% confidence interval of the mean. Shapiro-Wilk test of normality is used to test normality hypothesis of all quantitative variables for further choice of appropriate parametric and non-parametric tests. Mostly the variables are found normally distributed allowing the use of parametric tests. Independent samples t test is used for comparing the mean of two groups. For more than two groups one way Analysis of variance ANOVA test is applied followed by Bonferroni method for multiple comparisons. Significance level is considered at  $P < 0.05$  (S); while for  $P < 0.01$  is considered highly significant (HS). Two Tailed tests are assumed throughout the analysis for all statistical tests.

## **Results:**

### **1- Morphological examination:**

The outer surface of enamel stereomicroscopic assessment revealed white prenatal enamel with less translucency. Postnatal enamel appeared white in color with more translucency, in addition Neonatal line appeared chalky white in appearance, separating prenatal enamel near the dentio-enamel junction and postnatal enamel near the cusp tip as in figure (1).

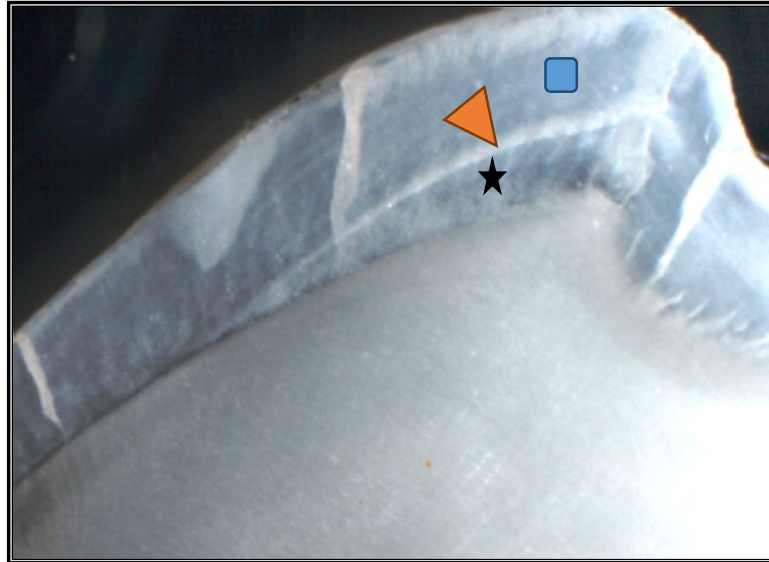
Light microscopic examination of longitudinal and transverse ground sections of DSM revealed dark accentuated neonatal line wide coronally at midway of the tooth separating prenatal from postnatal enamel, whereas the amount enamel formed at midway of tooth of prenatal enamel was greater than or equal that of postnatal enamel. In longitudinal ground sections, the neonatal line becomes thin as we go more cervically with amount of formed enamel at prenatally enamel was greater than that of postnatally as in figure (2 A & B).

### **2- Ultrastructure examination and morphometric analysis:**

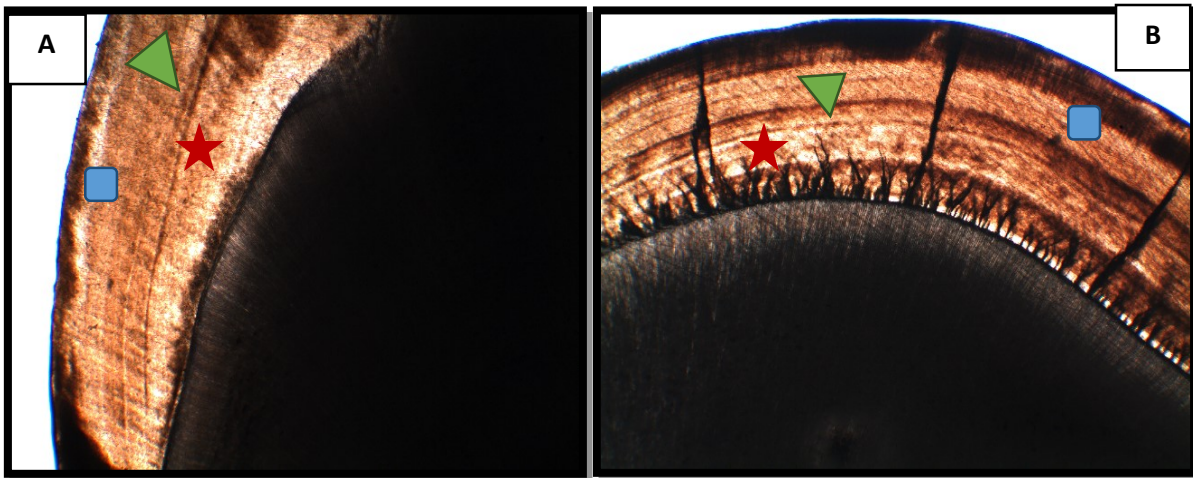
SEM results showed small beaded appearance of enamel rods of prenatal enamel and larger keyhole appearance in postnatal enamel. Neonatal line appeared radiolucent at site of changing the orientation of enamel rods as in fig. (3). The total number of prenatal enamel rods was larger than that of postnatal enamel rods with statistical significance. In contrary, the surface area of formed prenatal enamel rods was larger in postnatal enamel than prenatal enamel with statistical significance as shown in table (1 & 2) and fig. (4).

### **3- Chemical Analysis:**

EDXA mean value results of weight % of prenatal enamel showed a significant increase Ca and Ca/P ratio weight % with a significant decrease in C and non-significant decrease in P weight % when compared to postnatal enamel. Similar results were obtained when postnatal enamel was compared to neonatal line regarding Ca, P & Ca/P ratio weight % with a non-significant decrease in C weight %. Prenatal enamel showed a non-significant increase in Ca, P weight %, and Ca/P ratio and a significant decrease in C % when compared to neonatal line. Mg and Na weight % were absent in prenatal enamel. Neonatal line showed a significant increase in Na weight % than postnatal enamel. Postnatal enamel Mg weight % showed a non-significant increase when compared to neonatal lines shown in Fig. (5) and tables (3, 4 & 5).



**Fig. (1). A** photomicrograph of Stereomicroscopic examinationshowing prenatal enamel (black star), Neonatal line (orange arrow head) and postnatal enamel (blue square) (X 25)



**Fig. (2). A** photomicrograph of longitudinal (A) and transverse (B) ground sections showing accentuated dark neonatal line (green arrow head), prenatal enamel (red star), and postnatal enamel (blue square) brown striae of Retzius and dentino-enamel junction. Moreover, Enamel lamella and Enamel tuft were apparent in transverse section(X 100)

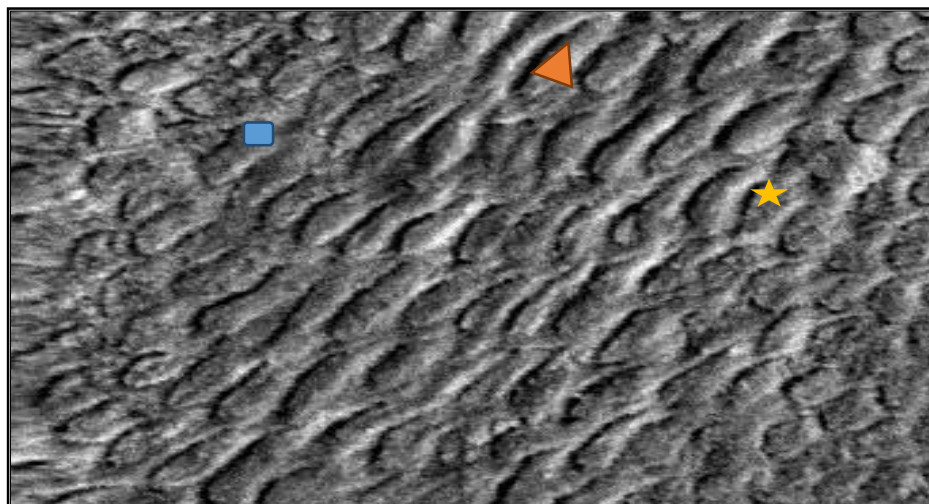


Fig. (3). A Scanning photomicrograph showing radiolucent neonatal line (orange head arrow), of enamel rods in both prenatal (yellow star) and postnatal enamel (blue square)(X1000)

Table (1): Difference In mean number of enamel rods per field between prenatal enamel and postnatal enamel using Paired Student's t-Test

Group	Mean number of enamel rods		
	Ms±Sd	t-Value	p-Value
prenatal enamel	140.00± 2.55	38.01	0.0001**
postnatal enamel	89.00± 1.58		

\*\*High Significant difference, (p<0.001).

Table (2): Difference In mean area% of enamel rods between prenatal enamel and postnatal enamel using Paired Student's t-Test

Group	Mean area% of enamel rods		
	Ms±Sd	t-Value	p-Value
prenatal enamel	63.7± 1.3	4.68	0.0002**
postnatal enamel	99.1± 2.2		

\*\*High Significant difference, (p<0.001).

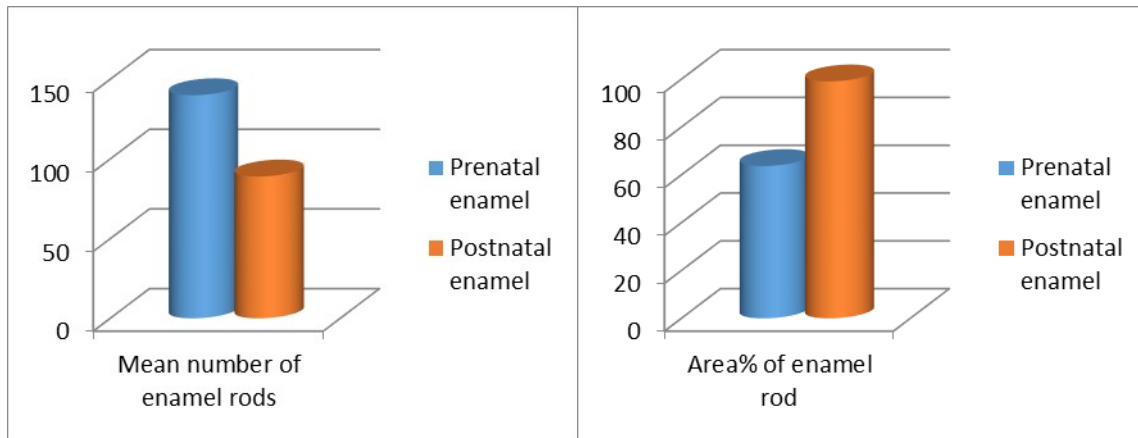


Fig. (4). Histogram II: Difference In mean number and area% of enamel rods per field between prenatal enamel and postnatal enamel.

**Table (3): ANOVA weight % of C, O, Ca, P and Ca/P ratio in prenatal, postnatal enamel and neonatal line.**

ANOVA WEIGHT %		N	Mean	SD	SEM	Lower Bound	Upper Bound	Minimum	Maximum	F	P value	
C wt%	Prenatal enamel	10	4.43	3.78	1.20	1.73	7.14	0.00	8.64	7.438	0.00267	<b>P &lt; 0.01 HS</b>
	post natal enamel	10	7.96	0.99	0.31	7.25	8.67	6.50	9.10			
	neonatal line	10	7.78	0.76	0.24	7.23	8.32	6.71	8.70			
P wt%	Prenatal enamel	10	17.02	0.83	0.26	16.43	17.62	15.80	18.00	1.703	0.20114	<b>P &gt; 0.05 NS</b>
	post natal enamel	10	16.67	0.19	0.06	16.53	16.81	16.54	17.01			
	neonatal line	10	17.06	0.30	0.09	16.85	17.27	16.67	17.52			
Ca wt%	Prenatal enamel	10	32.22	1.03	0.33	31.48	32.95	30.64	33.20	37.422	0.00000	<b>P &lt; 0.001 HS</b>
	post natal enamel	10	27.83	0.74	0.23	27.30	28.36	27.00	29.00			
	neonatal line	10	31.29	1.64	0.52	30.12	32.46	29.68	33.62			
Ca/P ratio wt%	Prenatal enamel	10	1.90	0.08	0.03	1.84	1.95	1.78	2.08	26.657	0.00000	<b>P &lt; 0.001 HS</b>
	post natal enamel	10	1.67	0.04	0.01	1.64	1.70	1.63	1.75			
	neonatal line	10	1.83	0.09	0.03	1.77	1.90	1.76	2.01			

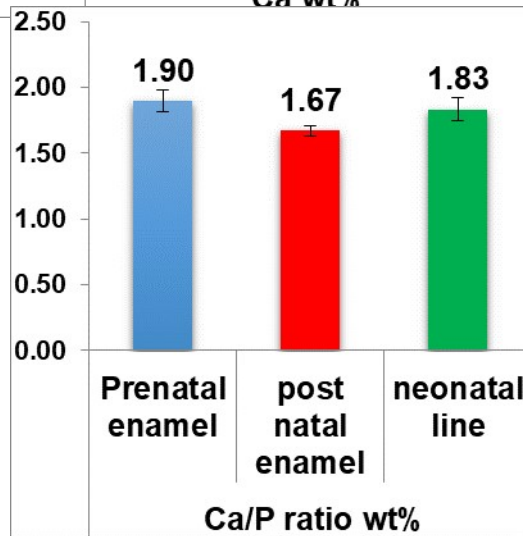
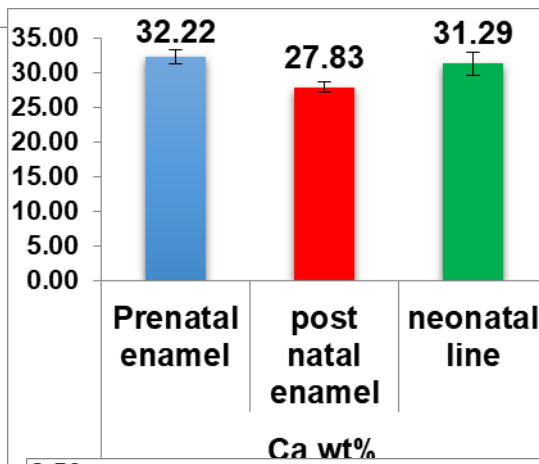
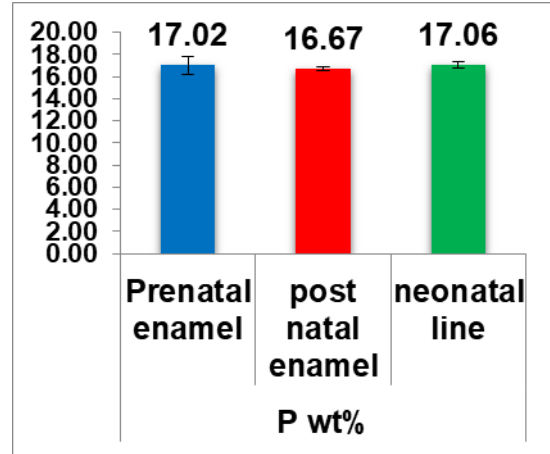
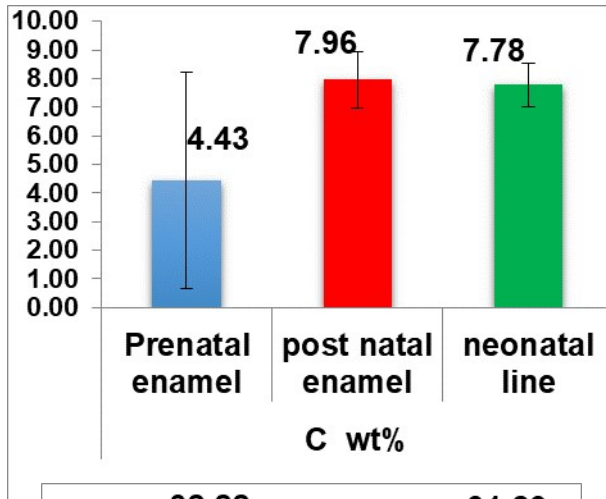
Multiple Comparisons Bonferroni Method wt%			Mean Difference	Std. Error	P Value	95% Confidence Interval		
						Lower Bound	Upper Bound	
C wt%	Prenatal enamel	post natal enamel	-3.52	1.03	0.00596	-6.15	-0.90	<b>P &lt; 0.01 HS</b>
	Prenatal enamel	neonatal line	-3.35	1.03	0.00925	-5.97	-0.72	<b>P &lt; 0.01 HS</b>
	post natal enamel	neonatal line	0.18	1.03	1.00000	-2.45	2.80	<b>P &gt; 0.05 NS</b>

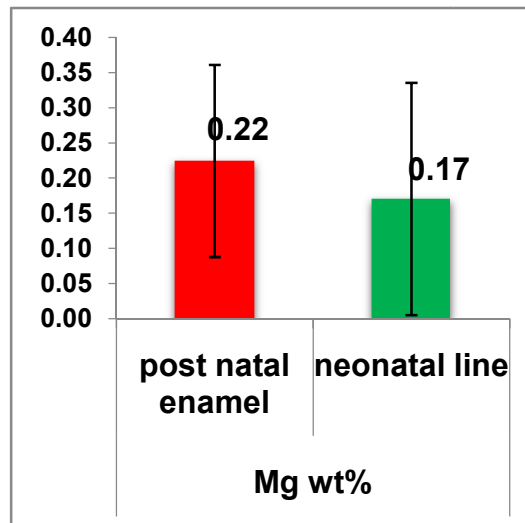
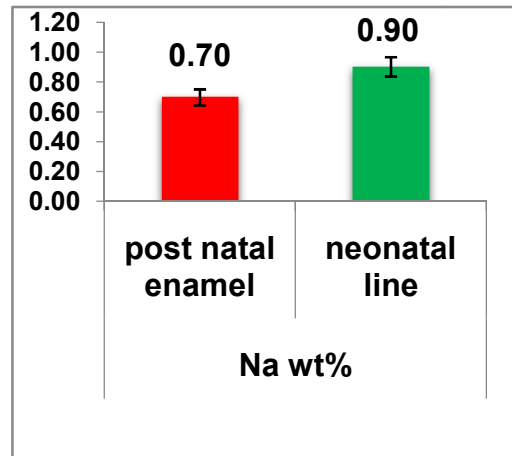
<b>P wt%</b>	Prenatal enamel	post natal enamel	0.35	0.23	0.42940	-0.24	0.95	<b>P &gt; 0.05 NS</b>
	Prenatal enamel	neonatal line	-0.04	0.23	1.00000	-0.63	0.56	<b>P &gt; 0.05 NS</b>
	post natal enamel	neonatal line	-0.39	0.23	0.31630	-0.99	0.20	<b>P &gt; 0.05 NS</b>
<b>Ca wt%</b>	Prenatal enamel	post natal enamel	4.39	0.53	0.00000	3.03	5.76	<b>P &lt; 0.001 HS</b>
	Prenatal enamel	neonatal line	0.93	0.53	0.28159	-0.44	2.29	<b>P &gt; 0.05 NS</b>
	post natal enamel	neonatal line	-3.46	0.53	0.00000	-4.83	-2.10	<b>P &lt; 0.001 HS</b>
<b>Ca/P ratio wt%</b>	Prenatal enamel	post natal enamel	0.23	0.03	0.00000	0.14	0.31	<b>P &lt; 0.001 HS</b>
	Prenatal enamel	neonatal line	0.06	0.03	0.19461	-0.02	0.14	<b>P &gt; 0.05 NS</b>
	post natal enamel	neonatal line	-0.16	0.03	0.00006	-0.25	-0.08	<b>P &lt; 0.001 HS</b>

**Table (4): Multiple Comparisons Bonferroni Method weight % of C, O, Ca, P and Ca/P ratio in prenatal, postnatal enamel and neonatal line.**

**Table (5): t-Test weight % of Mg, Na and Cl in postnatal enamel and neonatal line.**

		N	Mean	SD	SEM	Mean Difference	SE Difference	Lower	Upper	t	df	P Value	
<b>Na wt%</b>	post natal enamel	10	0.70	0.05	0.02	-0.21	0.03	-0.26	-0.15	-7.66	18	0.00000	<b>P &lt; 0.001 HS</b>
	neonatal line	10	0.90	0.07	0.02	-0.21	0.03	-0.26	-0.15				
<b>Mg wt%</b>	post natal enamel	10	0.22	0.14	0.04	0.05	0.07	-0.09	0.20	0.80	18	0.43578	<b>P &gt; 0.05 NS</b>
	neonatal line	10	0.17	0.17	0.05	0.05	0.07	-0.09	0.20				





**Fig. (5)Plate of EDXA statistical results graph of prenatal enamel, postnatal enamel and Neonatal line elements by weight%.**

**Discussion:**

Enamel is unique regarding metabolic disturbances during the time of tooth formation. Detailed knowledge of normal development and of the structure of enamel is important when assessing mineralization defects in order to preserve DSM in its place to preserve arch space and prevent malocclusion. The enamel of DSM develops partially before and partially after birth. (Leite-Cavalcanti et

**al., 2008; Mishra et al., 2009; Sabel, 2012 and Dantas et al., 2020**). Thus, the present study was designed to study the mineral content in prenatal, postnatal enamel and Neonatal line and its correlation to enamel morphology

LM of the present study in transverse section showed accentuated dark neonatal line. So that, prenatal enamel extended from the neonatal line to the dentino-enamel junction. In addition the postnatal enamel extended from neonatal line to the outer surface of enamel. In LM longitudinal section the prenatal enamel extended from neonatal line to the cusp tip. While the postnatal enamel extended from neonatal line to the cervical line. Moreover, the SEM results showed radiolucent neonatal line separating prenatal enamel rods from postnatal enamel rods. This occurs due to different crystal orientation between prenatal and postnatal enamel. These results were similar to that of **Berkovitz et al. (2017); Nava et al. (2017) and Dantas et al., (2020)**. However, the increased mean surface area of postnatal enamel rods in comparison with prenatal enamel rods. This could be due to that the amount of enamel formed after birth was more than formed before birth. This was supported by the study of **Sabel (2012)**, who claimed that teeth in the primary dentition started their mineralization during the gestational period and the last primary tooth is completed around 3–3.5 years of age. He also supported the increased mean number of prenatal enamel rods than that of postnatal enamel rods. In contrary, **Sabel et al., (2008)** stated that, the mean diameter of the prisms in the postnatal enamel was 0.55  $\mu\text{m}$  smaller compared with the diameters of the prisms in the prenatal enamel of all the tooth buds analyzed (1–19 months of age).

Stereomicroscopic examination of the present study showed postnatal enamel to be more translucent than prenatal enamel. In addition neonatal line appeared chalky white. These results were in agreement with **Berkovitz et al. (2017)** who suggested that deciduous teeth enamel formed prenatally is more opaque than permanent teeth enamel formed postnatally. This could be attributed to the decreased homogeneity of enamel rods formed prenatally in deciduous teeth than formed prenatally in permanent teeth. In contrary, the current study was not in agreement with **Thomas (2003) and Mishra et al. (2009)**, who stated that, prenatal enamel had apparent homogeneity in comparison to postnatal enamel and the increased opacity in postnatal enamel could be attributed to its re-mineralization by saliva and oral fluids intake.

EDXA results of the present study showed a significant increase in prenatal enamel Ca, P weight % and a significant decrease in amount of C weight % and absence of Mg and Na weight % in comparison to postnatal enamel. These results were in agreement with **Oliveira et al., (2010)** in Ca and P weight % of deciduous teeth formed prenatally. In contrary they reported that Ca and P weight % were higher in permanent teeth formed postnatally than in primary teeth formed prenatally. Moreover, **Bossù et al., (2020)** reported that deciduous teeth had major concentrations of Ca and P with minor concentrations of C and Mg. Comparing prenatal and postnatal enamel **Thomas et al., (2003)**, reported that prenatal enamel was highly mineralized than the postnatal enamel. The results were attributed to the fact that the prenatal enamel develops in protected surroundings with an adequate supply of all the essential nutrients. In contrary, **Mishra et al., (2009)** suggested that, that deciduous enamel is more susceptible to a carious attack than permanent enamel. This could be attributed to thinner thickness of deciduous teeth than the permanent ones, rendering it less mineralized (**Oliveira et al., 2010**).

In the current study, the postnatal enamel and Neonatal line decreased significantly than prenatal enamel in Ca and P weight % and increased significantly in C, Na and Mg weight%. However, neonatal line in the current study showed a significant increase in Ca, Ca/P ratio and Na weight% than in postnatal enamel. These results were in accordance to **Mishra et al., (2009)** who, suggested that the enamel formed at time of birth (neonatal line) was more mineralized than that formed either prenatally or postnatally. They explained that increased resting period allowed enamel to be formed slowly. This led to a decrease in carbonate-plus-magnesium ratio to calcium-plus phosphate rendering neonatal line hypermineralized. In contrary, **Birch & Dean, (2009)** and **Nava et al., (2017)** stated that neonatal line was hypomineralized. Regarding postnatal enamel of the existing study, the results were in accordance with **Liu et al., (2013)** in Ca, P weight %. They carried their study on permanent 3<sup>rd</sup> molars. The similarity between enamel development in primary teeth and that of permanent 3<sup>rd</sup> molars could be related to its late development and eruption. So that, permanent 3<sup>rd</sup> molars could represent the freshly formed postnatal enamel. However, the current study results were not in agreement with **Thomas et al., (2003)**; **Mishra et al., (2009)**; **Dean et al. and Dantas et al., (2020)** investigations who postulated that Postnatal enamel was commonly more highly mineralized due to postmaturation by saliva and dietary intake.

In the existing study C weight % showed a significant increase in postnatal enamel when compared to prenatal enamel and non-significant increase in postnatal enamel than neonatal line were in agreement with **Martinović et al., (2015)**. They stated that, Carbon concentration was significantly higher in hypomineralized enamel compared to healthy enamel.

In the present study Na weight % was absent in prenatal enamel. Neonatal line showed a significant increase in Na weight % than postnatal enamel. Little literature concerning Na weight % concentration was found in neonatal line. However, **Dean et al., and Dantas et al., (2020)** stated that, the low crystallinity (small crystal size) with inner crystalline disorders allows large surface area for absorption of ions and drugs to be re-mineralized, which explains the significant increase of Na in neonatal line when compared to postnatal enamel. In contrary, The results of the present study were not in agreement with **Hershkovitz et al., (2019)**, who found a significantly lower concentration of Na and Mg were in healthy prenatal enamel when compared to the prenatal enamel of cerebral palsy group. They also found that sodium in the prenatal enamel was significantly higher than in the postnatal enamel in both healthy and cerebral palsy groups. And this was explained due to narrow neonatal line observed in primary teeth of both healthy and cerebral palsy groups.

On the other side, concerning Mg weight% of the present study was absent in prenatal enamel but showed a non-significant increase in postnatal enamel than in neonatal line. This was supported by **Lagočka et al., (2003)**; **Teruel et al., (2015)** and **Klimuszko et al., (2018)** who reported that, Mg is an element that has an impact on the quality and anatomy of dental hard tissues. It is essential for the proper development of the tooth structure. Mg ions lead to the inhibition of the crystal growth by replacing calcium ions in hydroxyapatite. Moreover, Mg ions play a very important role in the regulation of hydroxyapatite crystal growth and determine physical and chemical stability of crystals. Mg may also affect the alkaline phosphatase activity which catalyzes the formation of appropriate hydroxyapatite crystals. Mg may inhibit the transformation of amorphous calcium phosphate to a crystalline form. Mg is also a component of the organic matrix of enamel. Additionally, **Dean et al., and Dantas et al., (2020)** conducted that, The presence of impurities in enamel crystals such as carbonate and Mg introduce significant stresses into the crystal structure which make it less stable and more reactive found in the existing study.

Furthermore, the existing study Ca/P ratio of postnatal enamel was in normal range and have more translucent appearance. Whereas, prenatal enamel was within the normal range and less translucent morphologically. Conversely, Neonatal line Ca/P ratio was within normal value with chalky white appearance. These results were supported by **Dorozhkin et al., (2009); Poorni et al., (2010); Klimuszek et al., (2018)** and **Arifa et al., (2019)** studies who postulated that normal enamel Ca/P ratio which ranges from 1.67 up to 2. In addition **Takagi et al., (1998) and Dorozhkin et al., (2009)** reported that, the Ca/P ratio weight% was related to the morphology of enamel rod crystals. Where, enamel crystal rods widen and thicken by additional growth with a simultaneous increase of Ca/P ratio and a decrease in carbonate content. In contrary, the results of the ongoing study postulate that Ca/P ratio doesn't play a great role in enamel homogeneity since, the degree of enamel translucency depends on enamel crystal lattice homogeneity. Thus, enamel homogeneity could be related to other elements in the hydroxyapatite enamel crystal lattice than Ca/P ratio. It could be related to presence of Mg which plays a role in crystal growth inhibition affecting enamel homogeneity or combination of many elements affecting the crystal lattice homogeneity (**Teruelet et al., 2015; Berkovitz et al., 2017; Klimuszek et al., 2018 Sfalcin et al., 2019**).

The present results, showed a significant increase in mean number of prenatal enamel rods than that of postnatal enamel rods. This result could explain the increased Ca/P ratio in prenatal enamel of the existing study. So that, the spaces between prenatal enamel rods were minimum which didn't allow remineralization by traces Na and Mg ions. Thus, the little amount of C weight% could be considered as an impurity rendering a high quality of enamel prenatally than that formed postnatally. The present study results of prenatal enamel showed homogenous less translucent white appearance. These results coincided with the existing study results which was supported by the significant increase in Ca, P weight % and significant decrease in C weight % with absence of Na and Mg weight %. These results could interpret that the high quality of formed enamel didn't allow infiltration of other impurities. However, the variation in concentration of elements could be related to different age groups or childhood illness during formation of enamel either prenatally or postnatally (**Alaluusua, 2010; Liu et al., 2013**).

### Conclusions:

1. Morphological study revealed that, postnatal enamel of DSM was more translucent and the enamel rods were more in surface area and less in number than prenatal. The neonatal line was chalky white appearance in stereomicroscope, accentuated dark line in LM radiolucent in SEM.
2. Elemental study revealed that, prenatal enamel of DSM is highly calcified enamel with maximum Ca/P ratio (1.9) with minimum impurities level of C weight % and less mineralized than postnatal enamel due to absence of Mg,
3. Postnatal enamel is more mineralized although it has less Ca/P (1.67) ratio than prenatal enamel, as it is remineralized by other traces of Mg. Postnatal enamel has more impurities with C weight % when compared to Neonatal line.
4. Neonatal line has Ca/P ratio (1.83) and is highly mineralized more than postnatal enamel with more traces of Mg% and more impurities of C weight %.

**References:**

- [1] Ahamed, S. S. S., Reddy, V. N., Krishnakumar, R., Mohan, M. G., Sugumaran, D. K., & Rao, A. P. Prevalence of early loss of primary teeth in 5–10-year-old school children in Chidambaram town. *Contemporary Clinical Dentistry* (2012); 3(1), 27.
- [2] Alaluusua, S. Aetiology of molar-incisor hypomineralisation: a systematic review. *European Archives of Paediatric Dentistry*, 11(2), 53-58.
- [3] Arifa, M. K., Ephraim, R., & Rajamani, T. (2019). Recent advances in dental hard tissue remineralization: a review of literature. *International Journal of Clinical Pediatric Dentistry* (2010); 12(2), 139.
- [4] Belikov, A. V., Altshuler, G. B., & Grishin, V. V. *Method and Apparatus for Tooth Rejuvenation and Hard Tissue Modification* (2008).
- [5] Berkovitz, B. K., Holland, G. R., & Moxham, B. J. *Oral Anatomy, Histology and Embryology E-Book*. Elsevier Health Sciences (2017).
- [6] Birch, W., & Dean, C. Rates of enamel formation in human deciduous teeth. In *Comparative dental morphology* (2009); 13, 116-120. Karger Publishers.
- [7] Bossù, M., Matassa, R., Relucenti, M., Iaculli, F., Salucci, A., Di Giorgio, G. & Di Carlo, S. Morpho-chemical observations of human deciduous teeth enamel in response to biomimetic toothpastes treatment. *Materials* (2020); 13(8), 1803.
- [8] de Andrade Dantas, E. L. D., de Figueiredo, J. T., Macedo-Ribeiro, N., Oliezer, R. S., Gerlach, R. F., & de Sousa, F. B. Variation in mineral, organic, and water volumes at the Neonatal line and in pre-and postnatal enamel. *Archives of Oral Biology* (2020); 118, 104850.
- [9] de Dios Teruel, J., Alcolea, A., Hernández, A., & Ruiz, A. J. O. Comparison of chemical composition of enamel and dentine in human, bovine, porcine and ovine teeth. *Archives of oral biology* (2015); 60(5), 768-775.
- [10] de Menezes Oliveira, M. A. H., Torres, C. P., Gomes-Silva, J. M., Chinelatti, M. A., De Menezes, F. C. H., Palma-Dibb, R. G., & Borsatto, M. C. Microstructure and mineral composition of dental enamel of permanent and deciduous teeth. *Microscopy research and technique*. (2010); 73(5), 572-577.
- [11] Dean, M. C., Humphrey, L., Groom, A., & Hassett, B. Variation in the timing of enamel formation in modern human deciduous canines. *Archives of oral biology* (2020); 114, 104719.
- [12] Dorozhkin, S. V. Calcium orthophosphates in nature, biology and medicine. *Materials*, (2009); 2(2), 399-498.
- [13] Hershkovitz, F., Shirley, L., Cohen, O., & Zilberman, U. The effect of cerebral palsy on neonatal line thickness and enamel components. *Archives of oral biology*, (2019); 104, 119-122.
- [14] Klimuszko E., Orywal, K., Sierpinska, T., Sidun, J., & Golebiewska, M. Evaluation of calcium and magnesium contents in tooth enamel without any pathological changes: in vitro preliminary study. *Odontology*, (2018); 106(4), 369-376.
- [15] Lagocka, R., Jakubowska, K., Lipski, M., Buczkowska-Radlinska, J., Chlubek, D., Opalko, K. The content of magnesium in superficial layers of human enamel and its influence on susceptibility of enamel to caries. (2003); *J Elementol*, 8:159–67.

- [16] Leite-Cavalcanti, A., Menezes, S. A., Granville-Garcia, A. F., &Correia-Fontes, L. B. Prevalence of early loss of primary molars: Study retrospective. *ActaSci Health Sci* (2008); 30(2), 139-43.
- [17] Liu, H. Y., Chao, J. H., Chuang, C. Y., Chiu, H. L., Yang, C. W., & Sun, Y. C. Study of P, Ca, Sr, Ba and Pb levels in enamel and dentine of human third molars for environmental and archaeological research. *Advances in Anthropology* (2013); 3(02), 71.
- [18] Macchiarelli, R., Bondioli, L., Debénath, A., Mazurier, A., Tournepiche, J. F., Birch, W., & Dean, M. C. How Neanderthal molar teeth grew. *Nature* (2006); 444(7120), 748-751.
- [19] Martinović, B., Ivanović, M., Milojković, Z., &Mladenović, R. Analysis of the mineral composition of hypomineralized first permanent molars. *Vojnosanitetskipregled* (2015); 72(10), 864-869.
- [20] Mishra, S., Thomas, H. F., Fearne, J. M., Boyde, A., & Anderson, P. Comparison of demineralisation rates in pre-and postnatal enamel and at the Neonatal line. *Archives of oral biology* (2009); 54, S101-S106.
- [21] Nava, A., Bondioli, L., Coppa, A., Dean, C., Rossi, P. F., &Zanolli, C. New regression formula to estimate the prenatal crown formation time of human deciduous central incisors derived from a Roman Imperial sample (Velia, Salerno, Italy, I-II cent. CE). *PloS one* (2017); 12(7), e0180104.
- [22] Poorni, S., Kumar, R. A., Shankar, P., Indira, R., & Ramachandran, S. Effect of 10% sodium ascorbate on the calcium: Phosphorus ratio of enamel bleached with 35% hydrogen peroxide: an in vitro quantitative energy-dispersive X-ray analysis. *Contemporary clinical dentistry* (2010); 1(4), 223.
- [23] Sabel, N., Johansson, C., Kühnisch, J., Robertson, A., Steiniger, F., Norén, J. G., & Nietzsche, S. Neonatal lines in the enamel of primary teeth—a morphological and scanning electron microscopic investigation. *Archives of Oral Biology* (2008); 53(10), 954-963.
- [24] Seow, W. Developmental defects of enamel and dentine: Challenges for basic science research and clinical management. *Australian Dental Journal* (2014); 59, 143–154. <https://doi.org/10.1111/adj.12104>
- [25] Sfalcin, R. A., da Silva, J. V. P., Pessoa, V. O., Santos, J., Olivan, S. R. G., Fernandes, K. P. S., Deana, A. M., Makeeva, I., Sauro, S., &Bussadori, S. K. Remineralization of early enamel caries lesions induced by bioactive particles: an in vitro speckle analysis. *Photodiagnosis and Photodynamic Therapy* (2019).
- [26] Takagi, T.; Ogasawara, T.; Tagami, J.; Akao, M.; Kuboki, Y.; Nagai, N.; LeGeros, R.Z. pH and carbonate levels in. developing enamel. *Connect. Tissue Res.* (1998); 38, 181-187.
- [27] Thomas, H. F., & Lee, D. Relative amounts of pre-natal and postnatal enamel in human primary incisors. In 32 nd Annual Meeting and Exhibition of the IADR (2003).
- [28] Zhang, J. Therapeutic effect of chitosan on remineralisation of enamel carious lesions by bioglass-based biomaterials (Doctoral dissertation, King's College London) (2018).



This work is licensed under a Creative Commons Attribution Non-Commercial 4.0 International License.