

Enamel Thickness in Primary Teeth

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Objective: The aim of this study was to determine the enamel thickness of all primary teeth in both maxilla and mandible. **Study Design:** An in vitro study was performed with fifty primary teeth including five each of central and lateral incisor, canine, first and second primary molars in both maxillary and mandibular region. Samples were mounted on wax sheets and scanned using a 64-slice CT scanner; which were then 3D reconstructed. Three serial slices were obtained from the middle of the coronal portion of each tooth. Volume rendering was done to differentiate three distinct zones of enamel, dentin and the pulp from each slice. A box was constructed touching the borders of the image on all the surfaces and the mid-point of each side was taken to measure the enamel thickness. **Results:** Tests used were ANOVA, Post-hoc Tukey's test and student's paired t-test. Enamel thickness was not the same on all the sides. Intergroup comparisons between maxillary anterior and posterior teeth showed difference in enamel thickness. On comparing the mandibular anterior and posterior teeth, the posteriors showed a greater value of enamel thickness on all the sides. ($p < 0.05$).

Conclusion: Primary enamel does not follow the same thickness patterns on all sides. It is also different in each primary tooth. There was an increase in enamel thickness in posterior teeth on comparison to their anterior counterparts in both maxilla and mandible. Enamel thickness was more on the distal aspect compared to mesial in all samples.

Keywords: Enamel thickness, Primary teeth, 64-slice CT scanner, Volume rendering, Tests, Intergroup comparisons

INTRODUCTION

In human teeth, development of enamel takes place in layers as secretory ameloblast cells migrate away from the underlying dentin towards the future outer surface and differentiate down along the enamel-dentin interface in a cervical direction. The enamel thickness of human teeth has not been studied much by the dental research workers except some occasional statement on measurements of specific areas.¹

The dental anatomy textbooks also do not make any reference to enamel thickness beyond the statement that the enamel is thickest at the incisal or occlusal surfaces and decreases in thickness gingivally.^{2,3}

The formation of enamel involves a rhythmic sequence of cellular activity interspersed with resting phases. Grine and Martin⁴ suggested that the quantity of enamel is the result of: (1) the rate of

enamel secretion, (2) the duration of enamel secretion, and (3) the number of enamel secreting cells.

The process of enamel formation, or amelogenesis, occurs predictably in tooth after tooth, generation after generation. The size, shape, shade, and even caries susceptibility of dental enamel can be passed from parent to offspring. Genetic diseases are associated with enamel malformations that range from total enamel agenesis to localized defects.

Thickness of enamel have provided important insights into primate taxonomic status and dietary adaptations.⁵ Moreover it is a vital parameter in various clinical procedures like proximal reduction, microabrasion, caries excavation, and pulp therapy and in research aspects involving caries, enamel demineralization/remineralization, and fluoride uptake. According to our knowledge only few studies had discussed about primary teeth enamel thickness.⁵ Furthermore, these studies were limited to examination of enamel thickness from histological thin sections of mesial or distal mandibular molar cusps. None of the studies had evaluated the enamel thickness of all primary teeth.

Various invasive techniques like physical sections,^{6,7} and noninvasive methods such as ultrasonic imaging,⁶ acoustic microscopy and radiography⁸ have been employed to measure enamel thickness. Other techniques like stereomicroscopy, scanning electron microscopy,^{9,10} flat-plane radiography,⁷ and most recently, X-ray computed tomography^{11,12,13} have also been tried with varying results. Computed tomography (using multiple X-ray detectors), allows to produce three dimensional reconstructed images of samples.¹⁴ Hence, we have used the latest, non-invasive and more accurate technique, the 64-slice CT, to measure the enamel thickness of all primary teeth.

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Figure 1. Stabilization and orientation of samples in wax sheet.

The aim of this study was to determine and compare the enamel thickness of all primary teeth in buccal, lingual, mesial and distal planes.

MATERIALS AND METHOD

The present study was carried out after approval from the Institutional Ethical Committee, Sree Balaji Dental College and Hospital, Chennai.

Fifty extracted primary teeth, which included five sets of central incisor, lateral incisor, canine, first molar and second molars from each arch irrespective of the sides were selected. Samples were under naked eye. Those teeth without any obvious enamel changes or fulfilling the following inclusion criteria¹⁰: minimum incisal or occlusal wear that is devoid of attrition, abrasion or erosion and absence of enamel defects, caries, fractures and restorations were chosen.

All samples were cleaned with a bristle brush and pumice. Samples were then stored dry¹⁰ in air tight containers away from sunlight until use. Each set of teeth was stabilized in separate wax sheets with the lingual surfaces facing the wax sheet and mesial side of each tooth facing the left side of the observer for the ease of identification. A metal scale was used to verify the parallelism of the mesio-distal axis and the right angle of the bucco-lingual axis of the teeth to the plane of the wax sheet (Figure 1).

A 64-Slice GE LIGHT SPEED CT Scanner (General Electronics, Light Speed VCT) was used for the study, which is capable of taking 64 slices of a tissue in one rotation with high resolution.

Each tooth was scanned with its vertical axis roughly perpendicular to the scanning plane and bucco-lingual axis parallel to the X-ray beam, and serial slices were obtained. Each section was reconstructed into an image of 512 x 512 pixels, with a pixel size of 0.028 mm. The serial slices of each tooth were compiled 3-dimensionally into a 512 x 512 x 512 matrix of isotropic voxels. The initial 512-matrix was reduced to a 256 x 256 x 256 matrix size for ease of measurement. This was done by averaging the gray scale values of adjacent voxels.

The 3-D reconstructed images of the teeth were obtained. For each sample, measurement of the inciso/occluso-cervical length was taken by drawing a straight line on the buccal aspect from the incisal or occlusal surfaces to the cervical margin by the computer software generated processing. For incisors, the mesiodistal distance was measured and a straight line drawn from the point bisecting this line (Figure 2a). In canines, the straight line was drawn from the cuspal tips to the cervical region (Figure 2b) and in molars the intercuspal distance was measured and the midpoint of this line was used to draw another straight line up to the cervical region (Figure 2c). In order to obtain the enamel thickness in the middle third of each sample, three points, point A, B and C were marked. Point A represented the mid-point of the straight line. Points B and C were marked at equal distances (0.5 mm above and below) from point A (Figure 2). Care was taken such that all these three points lie in the middle third of the crown. These three points were selected in the middle third of the crown because in primary teeth there is presence of contact areas between the molars rather than contact points as in permanent teeth.³ From these three reference points, 3D cross-sectional slices were obtained as gray scale images. By using a volume rendering software, contrast was provided to differentiate the four zones as enamel, dentin-enamel junction, dentin and pulp chamber.

An imaginary box was constructed touching the borders of the image on all the surfaces. On each side of the box, the midpoints of the line tangent to each surface of the teeth were taken as reference points. The enamel thicknesses were calculated in these reference points on all four surfaces (mesial, distal, buccal and lingual sides). This method was a modified version of the methodology used in a previous study with permanent mandibular incisors by Barrie Gillings.¹ The enamel thickness was calculated by measuring the distance between the outer enamel and the dentin-enamel junction (Figure 3).

All these scanning procedures and image analysis were performed by a single trained radiologist to avoid bias.

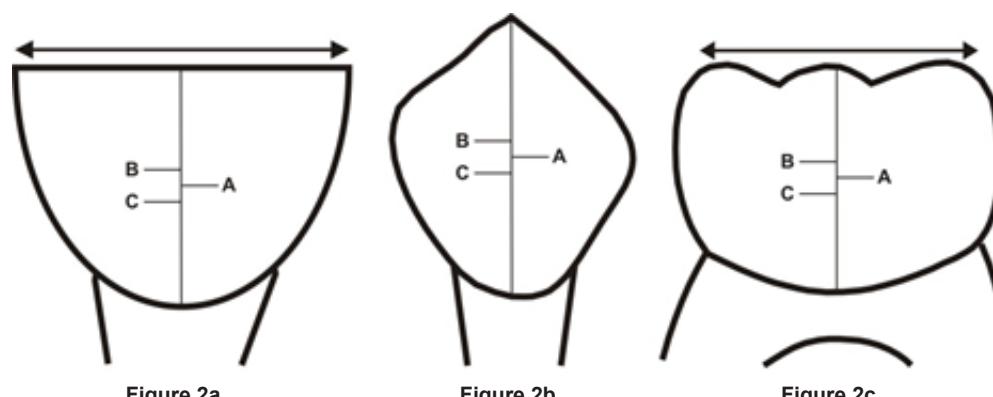


Figure 2. Calculation of the three reference points in incisors (figure 2a), canines (figure 2b) and molars (figure 2c).

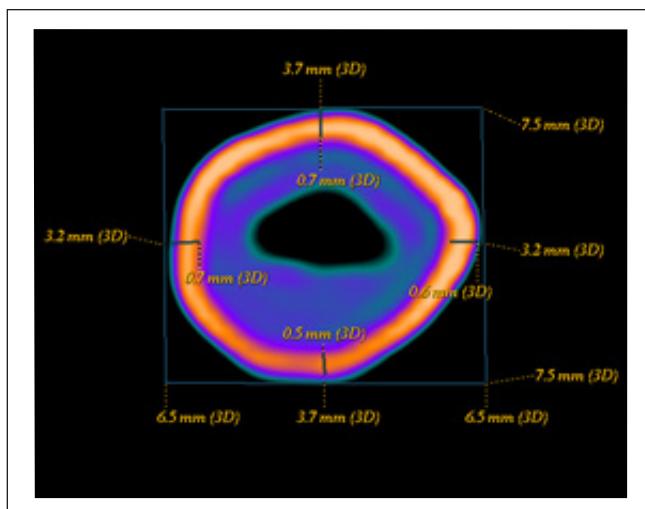


Figure 3. Cross-sectional view of one of the three serial slices, with a box reconstructed circumferentially and enamel thickness calculated on all four aspects

RESULTS

Data obtained were processed by SPSS software (12.0, SPSS Inc., Chicago I11, USA). A P-value less than 0.05 were considered as statistically significant. Independent samples t-test was used for comparing mean values of two groups. One way Analysis of Variance (ANOVA) was used to compare the mean values of more than two groups. Tamhane's post hoc test compared all possible combinations of the groups studied.

Inter-group comparison of mean enamel thickness in all the groups (Table 1)

All the groups were compared for the highest and the least values of enamel thickness in each side. The highest mean value of enamel

thickness on the mesial side was recorded in mandibular first molar and the least in maxillary lateral incisor. On the distal side enamel was found to be thickest in maxillary second molar and thinnest in maxillary lateral incisor, mandibular central incisor and mandibular lateral incisor which formed a single group. Maxillary first molar, maxillary second molar, mandibular first molar and mandibular second molar showed the highest value for enamel thickness on the buccal side, while maxillary lateral incisor showed the least. On comparing the lingual sides, it was found that maxillary first molar and mandibular first molar recorded the highest values of enamel thickness and maxillary lateral incisor the least. All the comparisons were found to be statistically significant using student's t-test, Tukey's post-hoc test and one way ANOVA.

Comparison of mean enamel thickness between the Maxillary and Mandibular Anterior and Posterior teeth (Table 2, Figure 4)

On comparison it was found that, though on the mesial side the values showed to be statistically non-significant but generally speaking the maxillary posterior teeth show greater enamel thickness than the maxillary anterior teeth. While comparing the mean values of enamel thickness between the mandibular anterior and posterior teeth, the mandibular posterior teeth show a significantly greater value on all the sides compared to the anterior teeth.

DISCUSSION

Enamel thickness is one of the most neglected aspects of dental anatomy. Studies are restricted to assumptions of enamel distribution patterns or the variations in enamel thickness in a single tooth and along the molar row.^{1,15} Furthermore, most of these studies have focused on the permanent dentition with fewer studies on primary teeth.¹¹ A study by Seow *et al*¹⁰ showed that, the thickness of enamel is reduced in preterm children, which also results in smaller crown

Table 1: The mean enamel thickness comparison of all the groups and intergroups (unit: mm)

Teeth	Mesial			Distal			Buccal			Lingual		
	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE
Maxillary Central Incisor (1)	.840	.130	.034	.940	.238	.062	.787	.168	.043	.713	.323	.083
Maxillary Lateral Incisor (2)	.607	.116	.030	.753	.168	.043	.600	.107	.028	.440	.150	.039
Maxillary Canine (3)	.767	.176	.045	.773	.158	.041	.667	.062	.016	.667	.123	.032
Maxillary First Molar (4)	.827	.194	.050	.907	.183	.047	.940	.135	.035	.973	.139	.036
Maxillary Second Molar (5)	.713	.141	.036	.960	.150	.039	1.033	.105	.027	.827	.175	.045
Mandibular Central Incisor (6)	.713	.141	.036	.733	.118	.030	.667	.098	.025	.587	.160	.041
Mandibular Lateral Incisor (7)	.700	.076	.020	.760	.124	.032	.653	.074	.019	.573	.122	.032
Mandibular Canine (8)	.780	.147	.038	.807	.128	.033	.687	.130	.034	.620	.190	.049
Mandibular First Molar (9)	.893	.122	.032	.867	.140	.036	.953	.099	.026	.893	.139	.036
Mandibular Second Molar (10)	.760	.135	.035	.820	.137	.035	.933	.199	.051	.653	.113	.029
Sig.	**			***			**			**		
Different Groups (based on Tukey's post hoc tests)	2 and 9; Others similar (1,3,4,5,6,7,8,10)			5 different; 2,6,7 one group; others similar (1,3,4,8,9,10)			4,5,9,10 – one group; 1, 2 different; Rest similar (3,6,7,8)			2 different; 4, 9 – One group; Others similar (1,3,5,6,7,8,10)		

** moderately significant ($P<.01$)

*** highly significant ($P<.001$)

ANOVA, Post-hoc Tukey's test

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Table 2: Comparison of mean enamel thickness between the maxillary and mandibular anterior and posterior teeth

Groups	N	Sides	Mean (mm)	P-Value
Maxillary Anterior	45	M	.7378	NS
		D	.8222	*
		B	.6844	***
		L	.6067	***
Maxillary Posterior	30	M	.7700	NS
		D	.9333	*
		B	.9867	***
		L	.9000	***
Mandibular Anterior	45	M	.7311	**
		D	.7667	*
		B	.6689	***
		L	.5933	***
Mandibular Posterior	30	M	.8267	**
		D	.8433	*
		B	.9433	***
		L	.7733	***

M, mesial; D, distal; B, buccal; L, lingual.

Student's paired *t*-test

* Significant ($P < .05$)

**Moderately significant ($P < .01$)

***Highly significant ($P < .001$)

dimensions. These facts led us to investigate the enamel thickness of individual primary tooth.

Physical cross-sections¹ of molar teeth have been used to measure enamel thickness by several workers. Physical sections provide an accurate portrayal of enamel thickness in a particular plane of section, but problems with specimen orientation (i.e., section obliquity) may render some of these data less than ideal. Moreover, because mechanical sectioning is destructive, as it is difficult to obtain adequate samples for statistical comparison.^{12,15} Additionally these sectioning will pose a problem in accurately reconstructing enamel cap volumes from physical sections.

Although non-invasive techniques like, acoustic microscopy⁸ and ultrasonic imaging^{6,16} have been used in several clinical studies, the accuracy of the quantitative data obtained has yet to be established.⁸

Lateral flat-plane radiographs have been used to measure enamel thickness in living humans.⁷ Standard bitewing radiographs of dental patients were used to study the effect of sex-linked genes on tooth size.^{15,18} These techniques only give qualitative data, that the enamel is thick in permanent molars and thin in primary molars. No definite quantitative data could be obtained.¹² Moreover, conventional radiography has the problem of superimposition and it is limited by the fact that the three-dimensional anatomy of the area being radiographed is compressed into a two-dimensional image.^{19,20} So measurements derived from radiographic images are unlikely to reflect the enamel thickness accurately.¹²

In the past two decades, Computed Tomography (CT) has found wide range of applications in dentistry.^{21,22,23} Because of its high resolution it gives a more detailed outlook of the internal structures,²⁴ but standard CT images generally exaggerate enamel thickness

compared to values obtained from mechanical sections.^{12,15} There are several reasons for this, including beam hardening artifacts, partial volume effect, and the improbability of obtaining a CT slice that traverses the tips of both dentine horns.²⁵ Technical advances in computer-assisted radiographic techniques have resulted in the application of a newer CT technique, the Spiral Computed Tomography.^{11,25} It has the inherent advantage of obtaining multiple slices in any plane with continuous rotation of the source detector assembly.²⁴ It is the newer generation of CT that has been used successfully in several endodontic research.^{16,21,22,23} Hence, we, in the present study have employed one of the latest non-invasive and more accurate technique, the 64-slice CT scan.

Gillings¹ examined the enamel thickness of permanent lower incisors and found that the enamel did not follow the same pattern on the buccal, lingual and proximal surfaces. The same pattern was also noticed in this study of primary teeth.

Intergroup comparisons among all the sides of maxillary central incisors, the buccal side showed the minimal enamel thickness of 0.787mm. The results of the present study were not in accordance with the study done by Seow *et al*¹⁰ where the enamel thickness on the facial aspect of primary maxillary central incisors of full-term was found to be 0.505mm. In a similar study done by Zuanon,²⁶ where he examined the maxillary primary central incisors, he found the thickness of buccal enamel to be 0.402mm before microabrasion.

Barrie Gillings,¹ in his study using permanent mandibular incisors found the buccal enamel to be the thickest of all surfaces, whereas in our study we found that the thickness of buccal enamel to be lower than the mesial and distal sides in mandibular incisors.

In a previous study done by Mahoney⁵ he used histological sections under transmitted light microscopy to determine the two-dimensional patterns of human enamel thickness on primary and permanent mandibular first molars; he found an increased tendency of average enamel thickness from anterior to posterior molars. His results were in contrast to our study, where we found, in maxillary segment, the maxillary first molars had more enamel thickness on the mesial and lingual sides than the maxillary second molars, while in the mandibular segment, and the first molars had more enamel thickness on all sides compared to mandibular second molars.

Stroud *et al*²⁷ did a study on enamel thickness in the permanent posterior teeth and found that the enamel thickness was consistently greater on the distal surface than on the mesial surface. This was in accordance to our study.

The posterior segments showed an increased enamel thickness in each arch, on all the mesial, distal, buccal and lingual aspects than anterior segments. This is in accordance with Mahoney's⁵ study. The much thicker enamel posteriorly might provide a stronger and more evenly distributed surface for grinding, as a result of a reduction of the lateral excursion of the mandible towards the temporomandibular joint.⁵

It has been previously reported in other studies that the Y-chromosome has a direct growth-promoting effect by influencing enamel formation compared to the X chromosome in individuals.¹⁸ In another study by Hall *et al*,⁸ predicting the variation in enamel thickness in mandibular incisors they found that black subjects had thicker enamel on average than did white subjects.

Hyun in 2009,¹⁷ did a study on thickness of primary enamel with known DLX3 mutation and found that the teeth affected by a 2-bp deletion in DLX3 has reduced enamel thickness compared to their normal counterparts.

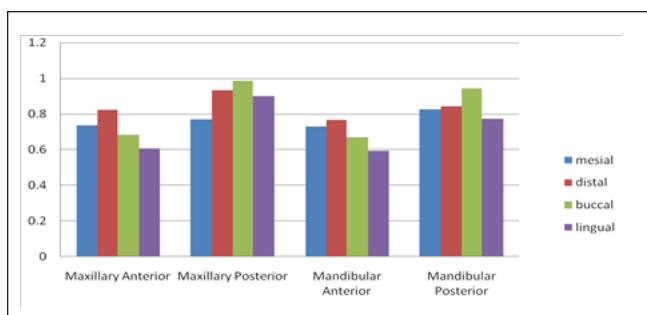


Figure 4. Graph showing intergroup comparisons of mean enamel thickness between the Maxillary and Mandibular Anterior and Posterior teeth (in mm.)

Therefore, the formation of dental enamel is somehow encoded in our genes, or DNA. So it is easy to assume that environmental and genetic factors play a major role in describing the enamel thickness patterns in different primary teeth; that dental enamel formation is under genetic control. Since the enamel is considered more of a mineral due to its rich mineral content; it has been reported by authors, that enamel formation is at least not directly encoded by gene. DNA can only encode RNA, and most of the RNA it encodes is used to make the specific proteins pertain to the dental enamel formation. Thus genetic control on enamel formation is more of indirect or at least not direct.

Some environmental factors are also discussed earlier by various authors like bite force,^{5,27} force direction,⁵ diet²⁸ and functional implications. Lucas,²⁸ had considered the role of diet as one of the important determinants in enamel thickness. Arguing the posterior increase in primary enamel thickness, Mahoney⁵ stated that it does not depend on the bite force. He and Stroud²⁷ considered the change in primary enamel thickness in terms of direction of force rather than the magnitude. Still it is unclear to what degree these differences are due to environmental or genetic factors.

Some of the shortcomings of this study are:

- Volume averaging artifacts of the 64-slice CT- As the pitch increases, the partial volume averaging increases thus making exaggerated effect of standard CT images.¹⁴
- The higher sensitivity and lower specificity of CBCT²⁴ imaging or rather the Micro-CT analysis¹⁹ can give better conclusive results.
- Finally, future studies can be done in this field with a larger sample size and in a diverse population.

Conclusions

- Primary enamel thickness pattern is dissimilar irrespective of the side or tooth.
- The maxillary and mandibular posterior teeth showed greater thickness of enamel on all the sides compared to their anterior counterparts.
- Enamel thickness was more on the distal sides compared to mesial in all teeth.

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