NO-FEE OPEN ACCESS JOURNAL indexed in Scopus

Bulletin of the International Association for Paleodontology

Volume 16, Issue 2, 2022

Established: 2007

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We thank all the reviewers for their effort and time invested to improve the papers published in this issue.

Enamel thickness of human mandibular canine: A radiographic study*

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Bull Int Assoc Paleodont. 2022;16(2):221-229.

Abstract

Enamel thickness of posterior mandibular dentition has been widely studied to explore the role of masticatory load in determining enamel pattern. Mesial-distal pattern of enamel thickness in posterior teeth is either a reflection of increasing magnitude of bite forces posteriorly or a developmental phenomenon. In the earlier sexual dimorphism studies, the thickness of enamel was more in females than males. However, research diverted to the importance of dentin in determining sexual dimorphism and its relation to the bite forces, with males showing greater dentin resulting in bigger teeth in them. This study had two objectives, one to examine the mesiodistal pattern of enamel thickness of mandibular canines and second to examine the sexual dimorphism in enamel proportion of mandibular canines. Crown width, mesial and distal enamel thickness, enamel cap area and tooth area were measured on digital periapical radiographs of mandibular canines of 85 subjects (44 females and 41 males) of Asian ethnicity using ImageJ. Mesial-distal enamel thickness was statistically analyzed by mixed factorial ANOVA and sexual dimorphism was assessed by logistic regression analysis. Enamel was significantly thicker on the distal than the mesial margins of human mandibular canines similar to the posterior dentition pattern. Sexual dimorphism was observed in enamel cap area as well mesial and distal enamel thickness with females showing more relative proportion of enamel than males.

Keywords: enamel thickness; sexual dimorphism; mandibular canines

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Introduction

Enamel and dentine are two dental hard tissues that primarily contribute to the thickness of crowns in all dimensions. Both the tissues differ in their origins as well as structure and composition, enamel is derived from the ectoderm and dentin from the neural crest (1,2). The thickness of enamel has drawn attention for decades. There is enough evidence on genetic regulation of enamel structure and thickness. Its functional role as the outermost covering of crowns of teeth also exerts influence on its thickness. Buccal- lingual comparisons of permanent molar enamel thickness have revealed thicker enamel on buccal cusps than lingual cusps (3,4,5). Mesial-distal comparisons of permanent molar enamel thickness report an increased distal average enamel thickness compared to mesial (6, 7). The thickness of enamel in general increases posteriorly in human molars which reflects an increase in bite force magnitude (biomechanical model of mastication (3,5,8,9). Alternatively, developmental aspects of enamel are also considered responsible for the thickness of enamel, in particular is the duration of enamel apposition. Increase in crown formation times is considered a key contributing factor in determining the mesial-distal patterns (5,10,11).

When it comes to sexual dimorphism of teeth, overall tooth size is bigger in males than females. This dimorphism is due to difference in enamel or dentin or both components of the tooth. Sexual dimorphism in teeth was initially attributed to differences in enamel content, with females showing more enamel (12). However later studies showed significant disparity in amounts of dentin, with more dentin in males than females leading to bigger crown dimensions in the former (1,2,13). Earlier, sexual dimorphism studies exploring enamel and dentin proportions, utilized linear measurements of enamel and dentin from periapical and bitewing radiographs (2,13,14,15). Later studies diverted to measuring area of these tissues on tooth sections and found area measurements to be more accurate sex predictors than linear measures (15,16,17). However these studies explored enamel cap area on tooth sections. This is a first study that explores sexual dimorphism in enamel cap area on digital periapical radiographs of human canines.

Studies investigating enamel thickness have given much attention to molars due to the important role they play in mastication (3,4,18). The anterior dentition in contrast, shows relatively scanty research on enamel thickness. The mandibular canine is the most sexually dimorphic tooth in human dentition and is generally well preserved even in fossil specimens (19). Our primary aim was to examine the mesial-distal pattern of enamel thickness of permanent mandibular canines on periapical radiographs and see if they show a similarity or variation to the mesial-distal patterns in posterior teeth reported in literature. Secondly, we explored sexual dimorphism in the enamel cap area of mandibular canines.

Materials and Methods

Digital periapical radiographs (radiovisuographs) of anterior teeth taken for diagnostic purpose were obtained from three clinics of south India. A ‘convenience’ sample was collected from centres that had informed consent from patients that allowed their data to be used for future research and hence did not require ethical approval. The sample consisted of 100 radiographs of persons of Asian ethnicity (50 females and 50 males) in the age group of 15-35 years. Lower limit of the age range was chosen to include fully mature canines and upper limit to avoid attrition that could possibly affect the enamel thickness. Cases with complete clinical records showing sound canines with no attrition or abrasion or orthodontic treatment, no history of bruxism were chosen. The enamel cap was identified as the most radiopaque region in the crown and only good quality radiographs with clear visualization of enamel were chosen and the radiographic images were not enhanced or adjusted. After careful exclusion the sample consisted of 85 radiographs (44, females and 41 males).

Sampling was conducted based on availability of records and no a-priori sample size calculations were performed. However, post-hoc examination shows that the sample size of 85 (44 females and 41 males) who were finally included in the 2x2 mixed-factorial ANOVA would result in an achieved power of 100% (within rounding error), 99.5% and 44.9% for large ($\eta^2 = 0.14$), medium ($\eta^2 = 0.06$) and small ($\eta^2 = 0.01$) effects respectively. Consequently the ANOVA can be said to be adequately powered to detect medium-to-large effects. These sample size calculations were done using G*Power (20).

Each radiographic image was imported to the software ImageJversion1.52a

(https://imagej.nih.gov/ij/download.html) and all measurements were performed by the first author. Since the radiographs were not taken from same machine and not standardized comparison of absolute measures was not possible. Hence ratios were used for statistical analysis.

Mesial-Distal comparison

The radiographic assessment of enamel thickness involved measuring enamel width and enamel cap area on each radiograph. To address our first aim of mesial-distal comparison of enamel width, three linear measurements were recorded using straight line tool of ImageJ, [crown width, mesial enamel width and distal enamel width] (figure 1). The crown width (CW) was measured perpendicular to the long axis of the tooth at the widest part of the tooth (the junction of incisal and middle third of the crown) (21). Mesial and distal enamel thickness was measured by a parallel line just below the crown width, from the DEJ to the mesial/distal outline of the crown. Ratio of mesial enamel width to crown width (ME/CW) and distal enamel width to crown width (DE/CW) were determined on an Excel sheet and only ratios were utilized for statistical analysis.

Sexual Dimorphism

Our second aim was to assess sexual dimorphism in enamel cap area and this was achieved by performing two types of measurements. Tooth area and enamel cap area were measured using polygon tool of ImageJ. (TA- entire tooth area with a free flowing line over the crown and root outline until you meet the start point; ECA- free-flowing line along the outer enamel outline of the crown internally over the DEJ till you meet the start point) (figure 3). Ratio of enamel cap area to tooth area was determined. Sex differences were also explored in the enamel width (EW) which was obtained by summing up mesial and distal enamel width (EW=ME+DE). Ratio of enamel width to crown width was determined and statistical analysis was performed on the ratios.

IBMSPSS22 software was used for statistical analysis. Descriptive statistics were sought for the pooled sample as well for individual sex groups and reliability was tested using Intraclass Correlations. Mesial-distal comparison of enamel thickness was analysed by paired 't' test on pooled sample and mixed factorial ANOVA across the two sex groups. Sexual dimorphism of enamel was assessed using binary logistic regression analysis performed on ratio of ECA to tooth area and total enamel width to crown width.

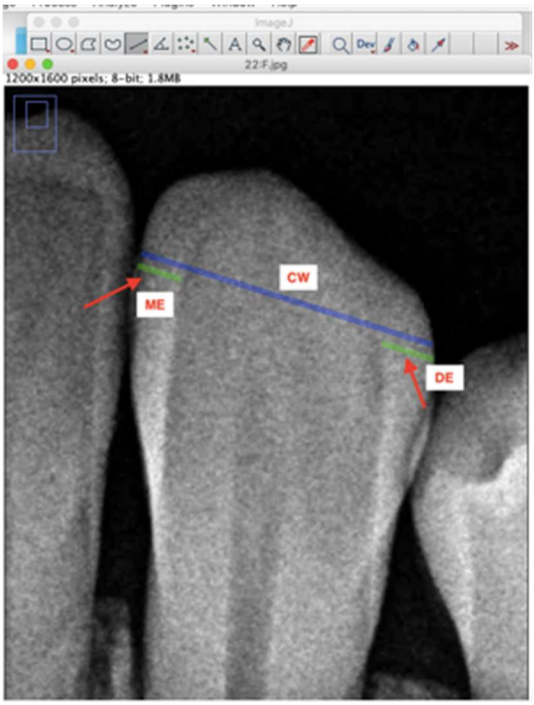

Figure 1. CW (blue line)- horizontal line drawn perpendicular to the long axis of the tooth*, at the junction of incisal and middle thirds of the canine in a mesiodistal plane; ME and DE widths (green line)- line drawn from the DEJ till the mesial and distal outline of the crown, parallel to and immediately below the CW.
*** In general, the long axis is defined as a two-dimensional imaginary line that passes through the cusp tip or the middle point of the crown and the apex or middle point of the root using dental or panoramic X-ray images (21).**

Results

Mesial-distal comparison

Descriptive statistics of crown width, mesial and distal enamel widths of the pooled sample are given in table 2. Paired 't' test on the pooled sample showed a significant difference between mesial and distal enamel width. Distally enamel was thicker than mesial side by 13.33pixels as seen in table 3. Analysis of Variance (ANOVA) on mesial and distal enamel ratios also showed that the enamel width was significantly greater on the distal aspect of the lower canine than mesial in both males and females. Females showed greater enamels width ratios (both mesial and distal enamel) than males as seen in table 4. Levene's test of equality of variances showed no problem with variances (table 5).

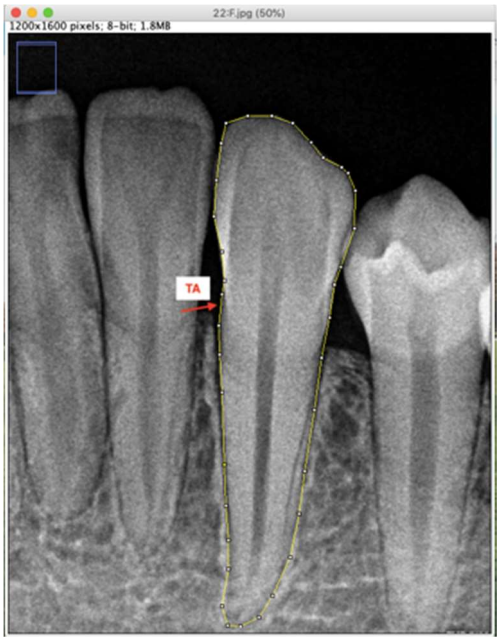

Figure 2. TA - entire tooth area with a free flowing line over the crown and root outline until you meet the start point.

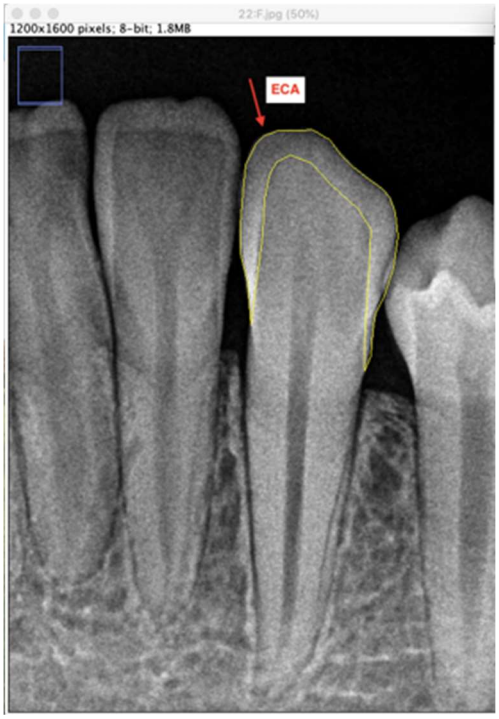

Figure 3. Enamel cap area (ECA) – free-flowing line along the outer enamel outline of the crown internally over the DEJ till you meet the start point.

Sexual Dimorphism

Descriptive statistics of all scores show the mean values of all measured variables of the lower canine (ECA, CW, ME, DE) are higher in males than females even though the radiographs were uncalibrated. However, when ratios were sought, enamel ratios were higher in females than males in all comparisons. Ratio of ECA/TA showed a significant statistical difference between the two sex groups and was higher in females than males. ME/CW, DE/CW and TE/CW, all enamel width ratios were also higher in females (table 6). Ratios here depict the tissue proportion in relation to the entire tooth. Hence the relative proportion of enamel in the overall tooth is higher in females. Logistic regression analysis was performed using ratio of ECA to TA for analysis of sexual dimorphism. The regression model showed a significant difference between the two sex groups indicating higher relative proportion of enamel within a female tooth than males. Another model was also tested using ratio of total enamel width to crown width. This model also showed a significant difference between the two sex groups.

Table 1. Abbreviations

CW	Crown width	Widest part of the tooth at the junction of incisal and middle third of the crown, measured perpendicular to the long axis of the tooth
ME	Mesial enamel width	Mesial enamel thickness, measured by a parallel line just below the crown width, from the DEJ to the mesial outline of the crown
DE	Distal enamel width	Distal enamel thickness, measured by a parallel line just below the crown width, from the DEJ to the distal outline of the crown
EW	Total mesiodistal enamel width	Obtained by summing up mesial and distal enamel width (EW=ME+DE)
TA	Tooth area	Entire tooth area measured with a free flowing line over the crown and root outline until you meet the start point
ECA	Enamel cap area	The most radiopaque region in the crown measured by a free-flowing line along the outer enamel outline of the crown and internally over the DEJ till you meet the start point
DEJ	Dentin-enamel junction	The interfacial region between the dentin and outer enamel coating in teeth.

Table 2. Descriptive statistics of crown width, mesial and distal enamel widths of pooled sample

	N	Min	Max	Mean	SD	Variance
CW	85	95.18	468.33	288.40	111.05	12333.55
ME	85	10.59	64.67	38.81	15.60	243.47
DE	85	14.32	106.19	52.14	22.17	491.56

N – number; Min – Minimum; Max – Maximum; SD – Standard Deviation

Table 3. Paired 't' test for pooled sample

	N	Mean	SD	Mean difference	t	df	2 tailed significance
ME	85	38.81	15.60	-13.33	-11.28	84	0.000
DE	85	52.14	22.17				

N – number; SD – Standard Deviation

Model using ECA ratio as the variable showed sex discrimination of nearly 70% of its utilized cases and model using enamel width ratio showed a 60% sex discrimination rate (table 7).

Reliability

Both intra and inter observer tests showed excellent single ICC suggesting high reproducibility of the method over time by the same expert and between different experts (Table 8).

Discussion

Mesial-Distal Comparison

The thickness of enamel shows variation in different teeth and within a tooth. In human mandibular molars, thicker enamel is found on buccal cusps than lingual cusps, in buccal-lingual comparisons, and distal margins than mesial margins, in mesial-distal comparisons. The thickness of enamel also increases posteriorly with more enamel in the third molars than the 1st molars. This variation in enamel thickness in mandibular molars is explained by a biomechanical model of mastication according to which, the mandible acts as a class III lever and the magnitude of masticatory forces increases posteriorly along the molar row. Hence increased enamel thickness in posterior teeth is a mechanism to adapt to higher bite forces. (5,22). Alternatively, a developmental reason also exists. Buccal cusp in a mandibular first molar takes longer to form than lingual cusp (23). Martin (11) stated that thicker enamel on the buccal cusps of a mandibular molar was due to increased enamel apposition time. Similarly, increased crown formation time was cited as a reason for increased enamel thickness on the distal permanent molar row (24). At the cusp tips, that serve as functional areas, mesially enamel was thinner raising speculation on the functional perspective in enamel thickness and reflecting on developmental reasons for mesial-distal variation. Hence thicker enamel may be due to increase apposition time resulting in more enamel cap area and thin enamel may be a result of slower enamel formation or cessation of ameloblast activity (5, 25).

The differences in mesial and distal enamel thickness have also been considered due to differential wear patterns. Mesial and distal movement of teeth over time contributes to interstitial wear. While both mesial and distal movements occur during mastication, mesial movement is more associated with eruption, pressure from soft tissues, occlusal forces and in particular the mesial tilt of teeth resulting in greater wear mesially than distally (26,27).

Our study showed that marginal enamel is significantly thicker distally than mesially in lower canines. Our findings support the earliest findings on mesial-distal patterns given by Shillingburg and Grace (28), Stroud and Buschang (27) and Harris and Hicks (2). The latter found the marginal enamel to be 0.1mm thicker on distal aspects of all upper incisors on periapical radiographs of maxillary central and lateral incisors. Stroud and Buschang also observed that enamel was significantly thicker distally than mesial in all mandibular posterior teeth ranging from 0.04mm to 0.19mm (27). Since our radiographs were uncalibrated, exact amount of difference in mm could not be ascertained. However, quite a significant difference was seen in the ratios.

The mesio-distal pattern of enamel thickness where distal enamel occupies a greater proportion of the mesiodistal width of the tooth than the mesial, is seen in both posterior as well as anterior teeth. Even though a clear relationship exists between enamel pattern and functional adaptation, it may not be the case in all segments of the jaws and all tooth aspects. Factors other than masticatory load, like developmental reasons and appositional times as discussed earlier, could be playing a role in determining mesiodistal thickness of enamel.

Table 4. Mixed factorial ANOVA for ME and DE ratios

Descriptives				ANOVA			
	N	Mean	SD	Factor	F (df)	p	Partial η^2
ME ratio	F (44)	0.14	0.02	Sex	8.891 (1,83)	.004	.097
	M (41)	0.12	0.02	Mesiodistal	190.514 (1, 83)	<.0001	.697
DE ratio	F (44)	0.19	0.03	Sex*Mesiodistal	1.613 (1,83)	.208	.019
	M (41)	0.18	0.03				

Table 5. Levene's test of equality of error variances

	F	df1	df2	Sig
ME ratio	0.054	1	83	0.817
DE ratio	1.252	1	83	0.266

Table 6. Descriptive statistics of enamel measurements of both sex groups

Variable	Mean		SD	
	Females (N=44)	Males (N=41)	Females	Males
CW	268.87	309.37	111.60	107.9
ME	38.61	39.03	16.57	14.69
DE	50.17	54.26	22.71	21.65
EW/CW	0.33	0.30	0.04	0.04
ME/CW	0.14	0.12	0.02	0.02
DE/CW	0.19	0.18	0.03	0.03
TA	217026.57	287390.00	142691.78	158197.65
ECA	31115.77	35619.95	20141.62	18956.133
ECA/TA	0.15	0.13	0.02	0.02

Sexual Dimorphism

Enamel width measured on periapical radiographs of maxillary central incisors and canines was more in females with 47, XXX than males and was substantially thinner in the absence of a second sex chromosome (45, XO). Conversely, males affected by Klinefelters syndrome with 47, XXY had much thicker enamel. These studies eventually indicate that X chromosome increases enamel growth (29,30,31). The ratio of enamel height and width to the crown-width was significantly larger in females and dentin-height and width to the crown-width was higher in males on bitewing radiographs of permanent mandibular 1st molars (32). When Schwartz and Dean measured both enamel and dentin areas from longitudinal sections of mandibular canines, they found more relative enamel thickness in females and greater dentin thickness in males (15). Similar results in enamel and dentin proportions were seen by Saunders et al on resin embedded sections of lower canines (17) and Monalisa et al on ground sections of maxillary and mandibular 1st premolar teeth (16). In contrast to these studies, enamel thickness between males and females showed no difference on human maxillary third molar sections (9), bitewing radiographs of mandibular premolars and molars (13), virtual sections of upper and lower canines (33) and periapical radiographs of upper incisors and bitewing radiographs of upper and lower deciduous molars (2). However all these authors relied on absolute measurements of enamel between the two sex groups.

Absolute size differences in crown dimensions or enamel thickness are very small in males and females and may be influenced by observer error (34). Even though absolute values of enamel thickness differed between maxillary molars, differences in distribution were similar. "How useful then is calculating overall enamel thickness for taxonomic purpose" is a speculation raised by Macho and Berner (9). Vice versa, our study showed increased absolute measurements of EW and ECA in males. However, the relative proportion of enamel within the tooth was higher in females. Perhaps relative measures of tooth components might prove more useful for taxonomic purpose. Since our absolute measurements were uncalibrated, we can only ponder on this finding without being overly conclusive.

A major factor that determines the thickness of the finished enamel is crystallite elongation at the secretory stage of amelogenesis. While amelogenin controls the growth and orientation of enamel crystallites, the influx of mineral for growth of these crystals is controlled by the ameloblast. Hence the thickness of enamel is determined by 2 components, amelogenin and ameloblast. Amelogenin, a major enamel protein accounting for 90% of extracellular matrix of enamel, is coded by genes on both X and Y chromosome. The mechanisms of alternative splicing of mRNA also differ between the two.

Table 7. Binary Logistic Regression Analysis

Predictor	Model 'p' value	% Sex correctly predicted in sample	Cox and Snell R square	Nagelkerke R Square
ECA/TA	0.001	69.4%	0.129	0.172
EW/CW	0.003	60%	0.097	0.129

Table 8. Intra and Interobserver reliability – ICC (2,1 – absolute agreement)

Variable	Intra-observer	Inter-observer
CW	0.997	0.972
ME	0.983	0.839
DE	0.960	0.862
TA	0.999	0.999
ECA	0.996	0.968

The end result is sexually dimorphic amelogenin proteins that are heterogenous in structure as well as function (35). A recent study showed immunohistochemical expression of estrogen receptor (ER α) in rat ameloblasts. High expression was seen in the secretory ameloblast than reduced ameloblast. The reaction was more cyclic and intense in ruffle ended ameloblasts than smooth ended ameloblasts corresponding with the cyclic process of enamel maturation. This indicates that estrogen signaling pathway is involved in enamel formation of rats, especially the mineralization process (36,37). Ferreti et al, showed that bone remodelling threshold in humans is possibly modified by estrogens leading to more bone mineral retainment in women than age matched men, eventually resulting in increased bone mass in premenopausal women (38). A similar parable could also exist in tooth. While amelogenin is determined in entirety by genes, ameloblast cell might be influenced by non-genetic factors like hormones, especially during mineralization. Hence, both genetic control of amelogenin in crystal growth and mineral regulation by ameloblasts under the influence of estrogens or related factors, can be contributory factors in the thickness of enamel. The fact that enamel dimorphism is seen in only certain tooth types could be due to periods of hormonal surges during development of only certain tooth types. It also explains the hypothesis of Alvesalo, seconded by Schwartz and Dean, that enamel growth may be regulated differently for different tooth types (14, 15, 29-31). On the other hand, sexual dimorphism in dentin thickness has been consistently seen in all tooth types, and in primary dentin even before puberty (32) indicating that dimorphism in dentin thickness may be primarily under genetic control.

Thus, strong genetic influence exists independently in both ameloblasts and odontoblasts, but these effects might be probably amplified during periods of hormonal surges, that might affect the mineral regulation of the cell, resulting in greater mineral content and greater tissue proportions of certain teeth only. The 'combined genetic and hormonal concept' requires further research on exploring estrogen receptors on ameloblasts and enamel organs, of both males and females, preferably on teeth showing highest sexual dimorphism. Examining relative proportions of enamel and dentin in different tooth types that develop during periods of hormonal surges and declines is another area that requires attention.

Conclusion

The pattern of enamel thickness on mesial and distal aspect of lower canines is similar to posterior teeth. Hence functional reasons alone may not govern the enamel thickness and developmental reasons also play a crucial role in determining the proximal enamel thickness of a tooth. Sexual dimorphism was seen in enamel, with females showing more relative proportion of enamel than males. Hence future sexual dimorphism studies on enamel and dentin might need to focus on relative proportions of tooth components than absolute measures.

Declaration of Interest

Funding: None
Conflict of Interest: None
Declaration of Interest: None

Author Contributions

Zama Moosvi: conceptualization, data curation, investigation, methodology, project administration, resources, software, visualization, validation, writing-original draft. Scheila Manica, conceptualization, methodology, software, supervision, validation, writing-review & editing. Gavin Revie, formal analysis, validation, visualization, writing-review & editing.

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