Sexual dimorphism of enamel area, coronal dentin area, bicervical diameter and dentinoenamel junction scallop area in longitudinal ground section

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Abstract

Introduction: Sex determination plays an important role in forensics; several studies done using radiographs, model cast and animal teeth have shown that males possess larger tooth crown and more dentin than that of the females. As physical sectioning of tooth provides more accurate measurement of enamel thickness when compared with other methods, the present study was done to evaluate and compare enamel area (EA), coronal dentin area (CDA), bi-cervical diameter (BCD), average enamel thickness (AET) and dentinoenamel junction scallop area (DEJ-SA) in longitudinal ground sections of first premolars between males and females.

Materials and Methods: A total of 60 extracted first premolar teeth were used for the study, of which 30 were from male and 30 were from female. A longitudinal ground section of 15 maxillary and 15 mandibular premolars of approximately 50 μm was prepared buccolingually at the center of each tooth and mounted on the slide. Multiple photomicrographs were captured with the help of Image Analysis System Software-Progres, Speed XT core 3. EA, CDA, length of the dentinoenamel junction and BCD were measured. Average DEJ-SA and AET were also calculated.

Results: Mann–Whitney U-test was used for statistical analysis. It was found that EA and AET were significantly greater in females than in males. On the contrary, it was found that CDA was significantly greater in males than in females. However, no significant difference was found in BCD and DEJ-SA between males and females.

Conclusion: Permanent first premolar can be reliably used in the field of forensic in establishing gender of individuals by measuring its EA, CDA and AET.

Keywords: Average enamel thickness, coronal dentin area, enamel area, ground section, sexual dimorphism

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INTRODUCTION

The teeth are the hardest tissue in the body and are extremely resistant to mechanical, chemical, physical and thermal destruction. They can endure even when whole body has undergone damage beyond recognition.[1-4] Teeth have an advantage in determining the gender even in a child

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where the skeletal features are not fully developed.[4] It also provides a reliable source for sex determination when the skeletal remains are insufficient or confusing. DNA investigation can also determine the gender accurately but is very expensive and time-consuming.[4] Gender can also be determined using craniofacial features, which when combined with odontometric features provide a better and more accurate identification. Hence, teeth prove to be an important element for anthropological, genetic and odontologic investigations in both living and dead people.[3]

Sexual dimorphism refers to systemic differences in size, height and form between males and females of the same species.[3,4] Since no two mouths are identical and the teeth are typical for males and females with respect to various features such as crown size, form, enamel and dentin thickness, pulp height and width, they can be applied to dental identification in gender assessment.[2,3] Gender determination is more crucial in identification of an individual as it halves the number of possible victims and once the gender has been established, it facilitates a more accurate identification of the deceased.[2,3,5]

Studies have been done using radiographs and medical computed tomography methods to measure the enamel thickness of tooth, but they do not provide an accurate measurement as they can either over- or undervalue the true measurement.[6-8] Radiographic images can give only visual idea of whether a tooth has thin or thick enamel. There are very few studies done with physical sectioning of teeth in a particular plane which gives a better and accurate measurement of enamel thickness compared to dental radiograph.[7,8]

Hence, the purpose of the present study is to evaluate and compare sexual dimorphism in enamel area (EA), coronal dentin area (CDA), average enamel thickness (AET), bicervical diameter (BCD) and dentinoenamel junction scallop area (DEJ-SA) in longitudinal ground section of first premolars among male and female. So far, no studies have been done on the measurement of DEJ-SA and depth.

MATERIALS AND METHODS

The study was conducted on extracted teeth, which were collected from the Department of Oral and Maxillofacial Surgery, and few teeth from the private dental clinics with permission from the concerned dentist and written consent of the patient.

A total of 60 extracted first premolar teeth were used for the study, of which 30 were from males and 30 were from females. Of the 30 in each group, 15 were maxillary and 15 were mandibular first premolars. The teeth were collected after obtaining written consent from the patients. The name, age and gender of the individual were recorded at the time of extraction of teeth. The teeth collected were washed thoroughly under running tap water and stored in air tight container with 10% formalin. Each sample was given a numerical code so as to blind the details of the sample. The inclusion criteria were permanent first premolar teeth indicated for extraction due to orthodontic reasons. The exclusion criteria were carious teeth, teeth with incomplete crown formation, enamel hypoplasia, cervical abrasion and teeth that show moderate to severe attrition.

Methods of preparation of ground section of the tooth

The tooth was mounted vertically in plaster of paris by covering half of the root length so as to hold the tooth firmly while sectioning. A line was drawn buccolingually/buccopalatally from buccal cervical line across center of the cusp tips to lingual cervical line by a permanent marker for reference [Figure 1]. With the help of carborundum disc attached to micromotor handpiece, the tooth was sectioned on both mesial and distal side of the reference line drawn on the tooth, so that a longitudinal section is obtained across the center of tooth [Figures 2 and 3]. Using an Arkansas stone, the tooth was further ground manually to a thin section of approximately 50 μm, polished, washed and mounted with DPX.

Methods of measurement

Mounted slide was placed on the stage of microscope in such a way that crown portion was directed towards the right side of examiner. The customized grid [Figure 4] was laid over mounted slide with one straight line of the grid aligned in line with the length of BCD.

In each prepared ground section, multiple photomicrographs of the tooth were captured using Olympus Research Microscope BX43F at 4× magnification. It was taken care

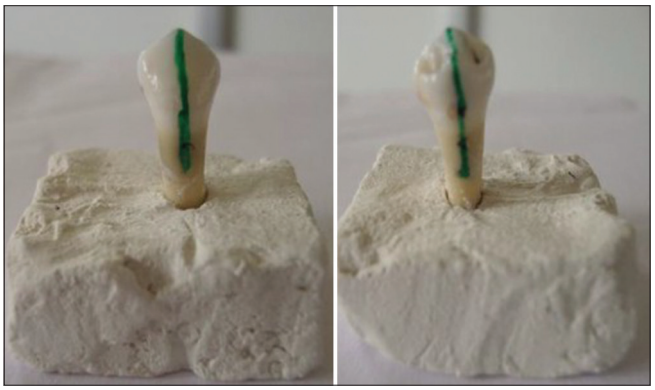

Figure 1: A reference line drawn buccolingually from the buccal to lingual cervical line and crossing the cusp tip

that each photomicrograph includes at least two rectangular grids. Photographs were captured starting from the upper cervical region and then proceeded downward till the lowest cervical region. Then, the field was shifted coronally to the next adjacent field and photomicrographs were captured as it proceeds upward till it reaches the uppermost region of the section. The field was then shifted coronally again to the next adjacent field and photomicrographs were captured in the same way as it proceeds downward [Figure 5]. Hence, multiple photomicrographs of the section were captured so as to involve the entire crown area. Then, the photomicrographs taken were segregated each for the EA, CDA and BCD. Measurements were done using Image Analyzer Software Progres SpeedXT Core 3 (JENOPTIK optical system GmbH, Germany). While measuring the dimensions, the upper and right side of the grid line were used to avoid error. In the sections of teeth that showed mild wearing of enamel, slight reconstruction was made during measuring by tracing an imaginary line to compensate wear.

Figure 2: The center part of the tooth after sectioning the mesial and distal portion of the tooth

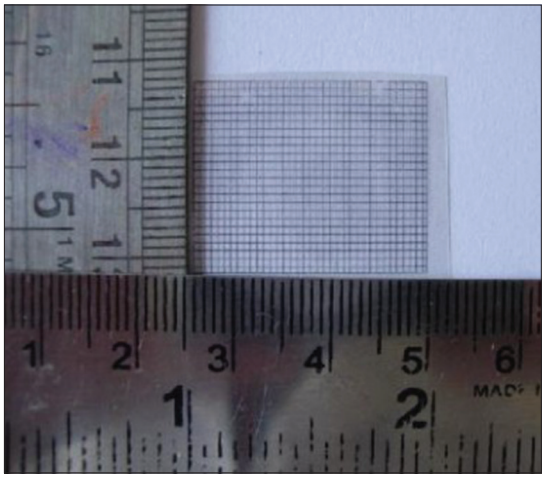

Figure 4: Customize graph on cellulose acetate sheet

Measurement of bicervical diameter

The distance between the two cervices represents the BCD which was measured by measuring its length across each grid along the length of BCD which was added up to obtain the total length of BCD [Figure 6a and b].

Measurement of the enamel area, dentinoenamel junction length and average enamel thickness

The individual EA within two rectangular grids were measured for each photomicrograph captured in enamel

Figure 3: The sectioned crown of the tooth after removing the mesial and distal portion of the tooth

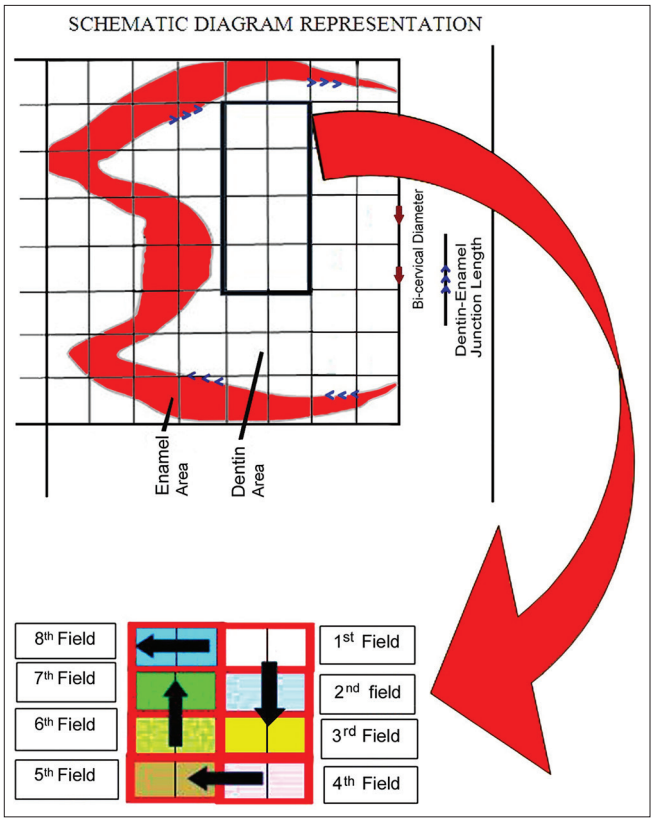

Figure 5: Method followed to capture all areas of section

region which were added up to obtain the total EA of the crown [Figure 7a and b]. The dentinoenamel junction length was measured by measuring along dentinoenamel junction in each grid and adding up the length [Figure 8a and b]. The dentinoenamel junction length was measured so as to calculate the AET.

The AET can be calculated as:

$$\text{AET} = \frac{\text{Enamel area}}{\text{Length of dentinoenamel junction}}$$

Measurement of coronal dentin area

Similar to the measurement of EA, the individual area of all rectangular grids in dentin region was measured which added up to obtain the total dentin area of the crown [Figure 9a and b].

Measurement of the dentinoenamel junction scallop area

The section was observed under 20× magnification and multiple areas showing clear dentinoenamel junction scallops were selected and captured. Area and depth of ten scallops in each section were measured and average value of the scallop areas calculated [Figure 10a and b].

The values obtained for EA, CDA, AET, BCD and DEJ-SA were tabulated and subjected to statistical analysis using Mann–Whitney U-test to compare between male and female mandibular first premolar and between male and female maxillary first premolar.

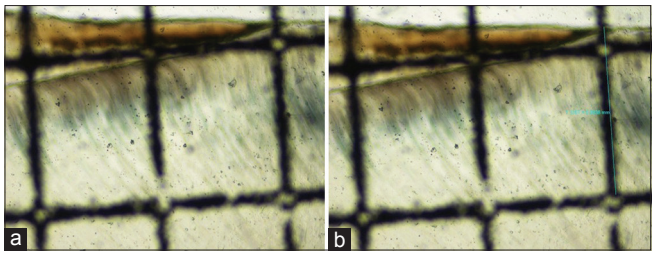

Figure 6: Photomicrograph of the prepared ground section showing the superimposition of straight line of the grid (a) and with measurement of bicervical diameter along the length of straight line of the grid (4×) (b)

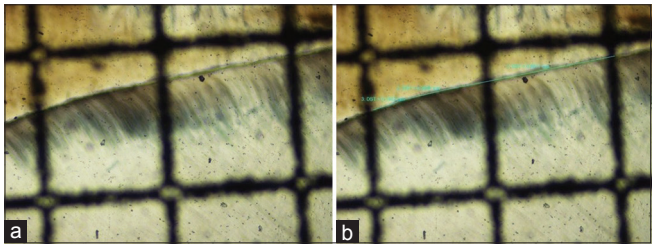

Figure 8: Photomicrograph of the prepared ground section showing the superimposition of two rectangular grids (a) with measurement of length dentinoenamel junction along the dentinoenamel junction in each grid (4×) (b)

RESULTS

Comparison between male and female first premolar

The EA, CDA, AET, BCD and DEJ scallop area were measured for both mandibular first premolar and maxillary first premolar. The values obtained were analyzed and compared between male and female using Mann–Whitney U-test. It was found that there was a statistically significant difference in EA, CDA and AET between male and female [Table 1].

EA and AET in both mandibular first premolar (P = 0.0001 and <0.00001, respectively) and maxillary first premolars ($P \leq 0.0001$ and < 0.0001, respectively) were found to be significantly greater in females than in males. CDA was found to be significantly greater in males than in females in both mandibular first premolar ($P = 0.0005$) and maxillary first premolars ($P \leq 0.0001$). However, BCD and DEJ scallop area showed no statistically significant difference between males and females in mandibular first premolar and maxillary first premolar.

DISCUSSION

The use of permanent first premolar teeth highlights the importance of situations when only posterior teeth are available for examination as anterior teeth are usually prone to be lost due to trauma and developmental anomaly.[9] Bharti A *et al*. also pointed out that since canine showed a highest degree of sexual dimorphism, the teeth neighboring to canine, that is, lateral incisor and first premolar showed

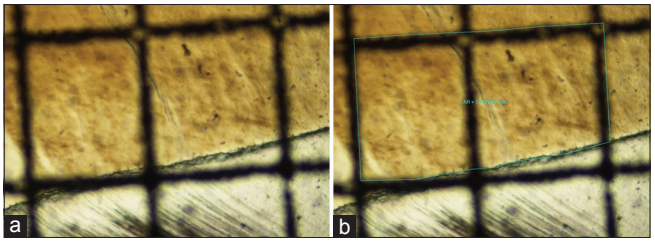

Figure 7: Photomicrograph of the prepared ground section showing the superimposition of two rectangular grids (a) with measurement of the area of two rectangular grids on the enamel area (4×) (b)

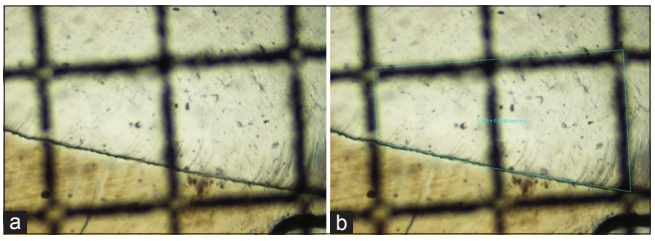

Figure 9: Photomicrograph of the prepared ground section showing the superimposition of two rectangular grids (a) with measurement of the area of two rectangular grids on the dentin area (4×) (b)

Table 1: Mann-Whitney U-test for the differences in enamel area, coronal dentin area, average enamel thickness, bicervical diameter and dentinoenamel junction scallop area

Characteristic	Male		Female		Mann-Whitney U-test	
	Median	SD	Median	SD	*Z* score	*P*
Mandibular first premolar						
BCD	6.47	0.53	7.07	0.79	−0.66	0.5
CDA	35.48	4.24	29.14	3.70	3.48	0.0005
EA	15.12	1.67	19.06	1.96	−3.87	0.0001
AET	0.92	0.049	1.09	0.11	−4.1	<0.00001
DEJ SA	0.00043	0.0001	0.00035	0.0001	1.099	0.27
Maxillary first premolar						
BCD	8.37	0.43	8.56	0.35	−0.2	0.833
CDA	42.81	4.13	37.73	2.34	4.23	<0.0001
EA	19.7	1.94	24.91	1.86	−4.23	<0.0001
AET	0.74	0.09	1.24	0.09	−4.64	<0.0001
DEJ SA	0.00043	0.0001	0.00047	0.0001	1.51	0.13

BCD: Bicervical diameter, CDA: Coronal dentin area, EA: Enamel area, AET: Average enamel thickness, DEJ SA: Dentinoenamel junction scallop area, SD: Standard deviation

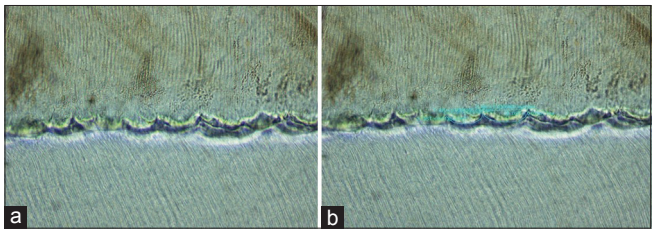

Figure 10: Photomicrograph showing the clear dentinoenamel scallop area (a) with measurement of dentinoenamel junction scallop area (20×) (b)

greater sexual dimorphism compared with the remaining teeth.[10] In the present study, we used permanent first premolar teeth because of this reason and also as it is frequently extracted due to orthodontic reason and such teeth showed least loss of enamel due to attrition or abrasion.

It was believed by many researchers that males possess a greater crown and dentin size than females due to a longer bell stage of tooth development when the dentin was deposited before the onset of amelogenesis.[2,11] Some authors have attributed this to the influence of human X and Y chromosomes. X chromosome is active in amelogenesis and promotes enamel formation, whereas Y chromosome promotes both the enamel and dentin formation.[12,13] Approximately 90% of the genetic coding for amelobin (the organic component, which constitutes 90% of enamel) is located on X chromosome with remaining 10% on Y chromosome in males.[14] Some researchers consider that changing levels of sex hormones during development could relate to the difference in proportions of dental tissues in teeth forming at different times.[6,9] However, this idea was not supported as a study done on mandibular second molar the crown of which develops during 3–6 years, showed a significant degree of sexual dimorphism in dentin area while there is not much role of sex hormones during the development of this tooth.[6] It was observed by some authors that the male teeth were heavier than that of females which may be due to the presence of more amount of dentin in the crown. This lead to the suggestion that dimorphism in tooth dimension is due to the difference in dentin proportion and not due to difference in enamel thickness.[6,15] Some investigators said that the variation in tooth size is also influenced by genetic, epigenetic and environmental factors such as nutrition, eating habits, disease and climate, and hence, there is a difference in sexual dimorphism of teeth between different populations and also between the generations.[9,16]

Results similar to our study have also been reported, done using several other methods such as radiographs, model casts and animal teeth. These studies reported that the crown and dentin size were much greater in males than in females, whereas females showed significantly thicker average enamel than in males.[2,6,7,11,14]

However, Harris and Hicks in their study stated that there is no distinct difference in enamel thickness between males and females after radiographic assessment of the enamel thickness in maxillary incisors.[11]

Though certain studies have shown that males possess a greater BCD than the females.[6,7] Our study showed no significant differences in BCDs between males and females similar to the study done by Johanna Morgan who concluded that mesiodistal and buccolingual cervical diameters are not a reliable indicator of sex determination.[14]

The measurements made from radiograph do not indicate accurate enamel thickness, instead, it may either under- or overvalue the true measurement. It gives only the visual idea of whether a tooth has a thin or thick enamel.[8] As many authors found that physical sectioning of teeth in a particular

plane of tooth gave better and accurate measurement of enamel thickness,[6-8] we used a ground section of tooth.

Many new advanced methods have also been established to determine the sex from the teeth using the medical computed tomography, examining trace elements in teeth and DNA techniques[17-19] and by measuring the concentration of Cobalt in dentin of teeth as Cobalt was found to be higher in women than in men.[20] Sex can also be identified by amplification of AMEL-X allele and AMEL-Y allele fragments of human amelogenin gene which is located on chromosomes X and Y.[19] However, these methods cannot be used all the time as they are highly technique sensitive and expensive and is not applicable in a large population, especially in mass disaster.

It was observed that in only 55.8% of the cases, it is possible to determine the sex based on craniofacial features but when combined with odontometric features, it is possible to determine sex in 86% of the cases.[16] Since most teeth complete their development before skeletal maturation, the dentition can be a valuable sex indicator, particularly in young individuals.[21]

Measurements of linear dimensions or odontometric parameters can be used for sex assessment in a large population because they are simple, reliable, inexpensive and easy to measure.[19] The emerging trend of forensic odontology in India relies a lot on inexpensive and easy means of identification of persons from fragmented jaws and dental remains. Although this method has its own merits, there are certain limitations. Preparation of ground section is a destructive procedure which uses up the entire tooth structure which may be required in situations where sample under consideration is inadequate or rare. It is indeed an undesirable technique when the subjects of the study are alive.

On the basis of the present study, it is concluded that the sex of an individual can be established from EA, CDA and AET of the maxillary and mandibular first premolar, which is an economical and a simple method. Further on, it is inferred that optimal results in dental sex assessment can be obtained when analysis of the first premolars are used along with other odontometric and skeletal traits. However, further studies have to be done in the evaluation of DEJ-SA since there might be a possibility that it may show some differences between males and females.

CONCLUSION

Studies have shown that there exists a correlation between sex and size of crown of the teeth after being measured on radiographs, medical computed tomography and model cast, with males possessing a greater tooth size than females. Thus it can concluded that the sex of an individual can be established from ground sections of enamel area, coronal dentin area and average enamel thickness of the maxillary and mandibular first premolar, which is an economical and a simple method. Yet, further studies have to be to be undertaken in the evaluation of dentino-enamel junction scallop area since there might be a possibility that it may show some differences between males and females.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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