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Morphology Analysis of Human Fourth Molars Using Computed Microtomography – Pilot Study

Ana Clélia Roussenq Baracho¹, Eun Sol Cho¹, Nayara Ramos de Almeida Torres¹, Thiago Gomes da Silva², Antonio Adilson Soares de Lima^{1,*}

¹ Departamento de Estomatologia, Universidade Federal do Paraná (UFRP), Curitiba, PR, Brasil ² Departmento de Engenharia Mecânica, Universidade Federal do Paraná (UFPR), Curitiba, PR, Brazil

* Corresponding author. Address: Curso de Odontologia, Departamento de Estomatologia da UFPR, Rua Prefeito Lothário Meissner, 632, Jardim Botânico, Cep: 80170-210, Curitiba, PR, Brazil. Phone: + 55 41 33604050. E-mail: aas.lima@ufpr.br

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Abstract. Analyze the morphological characteristics of fourth molars from non-syndromic patients using computed microtomography (microCT). Five human fourth molars (case group) and other three third molars (control group) were obtain from Biobank of UFPR Dentistry School. The teeth were submitted to macroscopic and microCT analysis. Sample characteristics: 60% of the teeth from the lower arch, mainly from the right lower arch (40%). The results showed that eighty percent of the teeth were impacted and all of them presented normal morphology. The microCT revealed that one of the fourth molars had hypercementosis. Concerning the root canal anatomy, all of the fourth molars studied had just one principal root canal. Four teeth had one collateral canal that ended at the lateral face of the root. The volume average of root canal, dentine and enamel were respectively 9.27 mm³, 122.64 mm³ and 74.71 mm³. The averages between these variables in third molars were 46.98 mm³, 473.25 mm³ and 198.34 mm³, respectively. Proportionally, the volume of the fourth molars was five times less than the third molars, but the dentine and enamel volume were, respectively, 3.8 and 2.7 times less than third molars. The fourth molar teeth usually showed only one principal root canal and it can be associated with other alterations, such as hypercementosis. Although the fourth molars were smaller, the proportion of enamel volume is bigger than third molar in comparison.

Keywords: Supernumerary tooth; Fourth molar; Hyperdontia; X-Ray Microtomography; Forensics.

1. Introduction

Forensic age and race estimation is an important element of anthropological research, as it produces one of the primary sources of data that researchers use to establish the identity of a person living or the identity of unknown bodily remains¹. The presence and characteristics of a supernumerary tooth may be a useful tool in human identification².

Once an ST is detected, its presence is documented in the dental records. ST are a unique entity such that its position, in relation to surrounding structures, is unique to a particular individual and becomes an important identification point in forensic odontology as it is always documented and can be compared with previous radiographs. There are numerous case reports which state the importance of identification of an individual based on the dental records such as radiographs, case sheets, study models, and supernumerary teeth³.

The permanent dentition is composed of 32 teeth among maxilla and mandibula jaws. Occasionally some individuals may have teeth in excess number than usual and these teeth are called supernumerary, which is a dental anomaly known as hyperdontia^{4,5}. According to Refoua *et al*⁶, it may result from horizontal proliferation or hyperactivity of the permanent or primary dental lamina. There are many theories about supernumerary teeth, but the etiology is still unknown. Hyperdontia is more frequent at permanent dentition⁵ and its incidence may vary between permanent and primary dentition in 0.1 a 2.1% e 0.3 a 0.8% respectively⁷⁻¹⁰. The supernumerary teeth may have normal form (supplementary teeth) or abnormal form (rudimentary teeth) such as conical shape or may be a dental tissue mass⁹.

Studies have revealed that supernumerary teeth are more frequent on upper teeth and among male individuals¹¹⁻¹⁶. Hyperdontia can be classified according to its location. Central incisors supernumerary teeth are called mesiodens; when they appear on molar region they are classified as paramolars; and those distal to the third molar as distomolars^{8,17}. Supernumerary teeth can cause eruption disorders, dental crowding, tooth reabsorption and odontogenic cists and tumors¹⁸.

Hyperdontia diagnosis is generally based on x-ray exams by counting the number of teeth. Panoramic x-ray is the ideal technique to find fourth molars since they are often impacted due to lack of space¹⁹⁻²¹.

Other x-ray techniques may help the diagnosis, like Miller-Winter, Donovan and Parma. Besides, the tomography can help to elucidate possible doubts about form and position of the fourth molars²².

MicroCT images are useful tools in assessment of age of an individual²¹ and a non-destructive analytic procedure that is able to characterize samples by determining their morphology and analyzing their structural properties²².

The objective of this pilot study was to evaluate the morphological characteristics of human fourth molars through computer microtomography (microCT).

2. Materials and methods

This study was submitted and accepted by the Ethical Committee of the Federal University of Paraná (UFPR) through the approval number 1.618.336 (Attachment1). The sample was composed of 10 human teeth divided into two groups: 5 fourth molars (case group) and 5 third molars (control group). Third molars were chosen as control group due to their similar anatomy. As inclusion criteria, preserved human teeth diagnosed as third and fourth molars were included in the study. As exclusion criteria teeth with any damage from surgical removal drill or enamel defects were excluded.

One fourth molar from three different individuals and two fourth molars from another individual were used. All participants agreed with the donation to the biobank and signed the legal terms.

All the sample teeth extractions were performed at the Surgical Clinic of UFPR Dentistry School. After the surgical procedure the specimen were immersed in a saline solution and then cleaned to be steam sterilized by autoclave. University's Biobank collected data as gender, age and systemic health from the patients' files.

The specimens were taken to Rocks and Minerals Laboratory (LAMIR) located on UFPR campus to be microCT analyzed.

Each group were placed in a polystyrene tray and scanned by Skyscan 1172 (Skyscan, Kontich, Belgium) to capture images using 90kV capacity and 112 μ A supply chain. The pixel configuration was 12.89 μ m without any filter with the exposition time of 1100ms per image, resulting in images with a 1,336 x 2,000 pixels resolution. After every image capture, the scan source turned 0.4° and this process

kept repeating until the sample tray turned 180° from its original position. Overall time of image capture was 35.48 minutes per group.

The tomography images were reconstructed using the NRecon program (Skyscan, Kontich, Belgium) to obtain the tomography slices in a perpendicular direction from the original one.

The images were then processed by the software CTAnalizer, CTAn (Skyscan, Kontich, Belgium) where the slices were converted into black and white images and separated in gray shades from 0 to 255, in the process of image binarization. Different density materials are contained in different gray shades what make possible to identify enamel and dentin to determinate the total volume of each one. After the binarization, the rendering process was elaborated to 3D image construction and posterior acquisition of volume quantity parameters. The rendered images were observed on a software (CTVol Skyscan, Kontich, Belgium) that produced a 3D model on Data Viewer (Skyscan, Kontich, Belgium) that allowed the visualization of the three axis at the same time. These 3D images were used to evaluate teeth internal anatomy and enamel and dentin volume.

One fourth molar was randomly chosen to illustrate the microCT exam in high definition with the following pattern: no filter, 4.32 μ m pixel, 2200 x 2672 px camera resolution, 700 ms time of exposure, rotation of 0.25° per image until 180°. Total time of image acquisition was 55.36 minutes.

Initially the root volume was analyzed since its x-ray absorption rate is different from the rest of the tooth, followed by the root tissue analysis that wouldn't contain enamel, only dentin. When the coronal portion was examined, where enamel and dentin coexist, it was necessary to make a binarization to distinguish the two structures that have very different x-ray absorption rates. Finally, the enamel volume was isolated to allow the sum of the total volume of dentin.

The statistical treatment was composed by descriptive analysis under mean and standard deviation form.

3. Results

Table 1 presents the clinical characteristics of the teeth donators and shows a gender balance. The age average of the individuals who donated the fourth molars when they were removed was 21.6 years old. Although 5 fourth molars have been collected, two came from the same individual. The majority of fourth molars were

from upper jaw (60%) and 40% from the right side of mandibula. Eighty percent were impacted and 100% had normal morphology.

Table1. Distribution of the fourth molars according to age, gender and localization.					
Fourth	Gender	Age	Localization	Eruption status Form	
molars					
Specimen 1	Male	23	Upper left	Unerupted	Normal
Specimen 2	Male	21	Lower right	Unerupted	Normal
Specimen 3	Female	24	Upper right	Erupted	Normal
Specimen 4	Female	20	Lower left	Unerupted	Normal
Specimen 5	Female	20	Lower right	Unerupted	Normal

Following the microCT analysis it was observed that one of the fourth molars had hypercementosis (Figure 1).



Figure 1. Fourth molar clinical characteristics with hypercementosis and its tomography image.

Regarding the root, canal system anatomy the microCT revealed that all fourth molars showed only one main canal and four of them had a collateral canal discharging into the lateral wall of the root (Figure 2). In concern to the third molars, one of the specimens had three root canals. Contrariwise, the other third molars had only two root canals and one of them showed a deformation at the apical portion and accessory canals.



Figure 2. All of the fourth molars showed a single main root canal and four of them at least one collateral canal discharging laterally.

Table 2 shows the values of root canal volume, dentin an enamel of the fourth molars. The average of root canal, dentin and enamel volume were, respectively 9.27 mm³, 122.64 mm³ and 74.71 mm³ (Table 3). The means of these variables were, in the same order, 46.98 mm³, 473.25 mm³ e 198.34 mm³. Correspondingly, the volume of the fourth molars was 5 times smaller comparing to third molars. As for dentin and enamel volume, the values were 3.8 and 2.7 times smaller than the third molars' volume.

Fourth molar	Canal (mm³)	Dentin (mm ³)	Enamel (mm ³)
Specimen 1	15.25	142.18	107.82
Specimen 2	10.07	111.02	75.01
Specimen 3	6.89	173.76	64.50
Specimen 4	6.37	91.61	63.58
Specimen 5	7.75	94.64	62.65
Mean	9.27	122.64	74.71
Standard deviation	3.62	34.91	19.16

 Table 2. Results of fourth molars volume analysis.

Table 3. Results of third molars volume analys

Third molars	Canal (mm ³)	Dentin (mm ³)	Enamel (mm ³)
Specimen 1	40.88	573.09	221.15
Specimen 2	43.41	363.63	158.27
Specimen 3	56.64	483.03	215.60
Mean	46.98	473.25	198.34
Standard deviation	8.46	105.06	34.80

The percentage of the volume of root canal, dentin and enamel was calculated and presented in Tables 4 and 5. The average percentage of these

variables on the fourth molars were, respectively, 4.5%, 59% e 36.5%. For the third molars the values were 6.7%, 65.6% e 27.7%, respectively.

Table 4. Results of fourth molars volume analysis in percentiles.				
Fourth molar	Tooth total	Canal	Dentin	Enamel
	volume (mm)	(%)	(%)	(%)
Specimen 1	265.26	5.74	53.60	40.64
Specimen 2	196.11	5.13	56.61	38.25
Specimen 3	245.16	2.81	70.87	26.31
Specimen 4	161.58	3,94	56,69	39.35
Specimen 5	165.06	4.70	57.33	37.96
Mean	206.63	4.50	59.00	36.50
Standard deviation	46.88	1.13	6.78	5.79

Table 5. Results of third molars volume analysis in percentiles.

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Fourth molar	Tooth total volume (mm ³)	Canal (%)	Dentin (%)	Enamel (%)
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Specimen 1	835.13	4.89	68.62	26.48
Specimen 2	656.33	7.68	64.32	27.99
Specimen 3	755.27	7.49	63.95	28.54
Mean	748.91	6.70	65.60	27.70
Standard deviation	89.56	1.55	2.59	1.06

It can be identified that the third molars percentage of volume of the three different structures (root canal, dentin and enamel) was higher than what was found on the fourth molars. Taking the fourth molars into account, the percentage of enamel volume in relation to the total volume was higher than in the third molars.

4. Discussion

Age and race estimation are common tasks in Forensic Dentistry. Dental structures are the hardest and well protected structures in the body. These structures resist decomposition and high temperatures and are among the last ones to disintegrate after death. Dental features such as tooth morphology, number of teeth, variations in shape and size, restorations, pathologies, missing tooth, wear patterns, crowding of the teeth, color and position of the tooth, rotations and other peculiar dental anomalies give every individual a unique identity. In absence of ante-mortem dental records for comparison, the teeth can help in the determination of age, sex and race, which can give further clues regarding human identification^{1,2,20}. The presence of a

fourth molar can be a key factor for individual recognition and understanding its morphology may help to identify age and sex.

This study investigated the morphological characteristics of fourth molars trough microCT images. Although a small sample was analyzed, the pilot study results showed that the fourth molars may have further alterations such as hypercimentosis. Besides, the microCT revealed that in regard to the internal anatomy, the fourth molars showed only one main root canal but presented collateral canals.

Odontogenesis is the complex process of tooth development and it has been characterized with a series of reciprocal interactions between epithelial and mesenchymal cells leading to tissue differentiation¹⁹⁻²². Teeth are formed initially as appendices of epithelia and its morphogenesis is regulated by the integration of epithelial and subjacent neural crest from mesenchyme. Mammal's dentition consists on a group of very specialized teeth known as incisors, canines and molars, which come from different areas of the oral epithelia¹⁹.

Hyperdontia is the term used to describe extra or supernumerary teeth that exceed the 20 deciduous teeth or 32 permanent teeth²³. Atavism, by definition, is the reappearance in an individual of characteristics of some remote ancestor²⁴ and it supports some theories for fourth molars development. Hyperdontia may occur due to a division of tooth germ and also as a consequence of genetic and circumstantial factors²⁵⁻²⁸. However, according to some studies²⁹ the final development post permanent dentition arises on account of late dental lamina conception, occurring after the permanent dentition is concluded.

In addition, the morphological analysis of the fourth molars the microCT also enables the evaluation of root canal, dentin and enamel. This technique was previously used by Núñez *et al*³⁰ to measure the volume and thickness of mineral density of rats' incisors enamel. Until the present day it couldn't be found similar studies to be compared to this one. Meanwhile, it was possible to calculate the percentage of volume occupancy of root canal, dentin and enamel and when its values were compared to the control group it was observed that third molars had a bigger value. Moreover, although one of the fourth molars had microdontia, the percentage of enamel in relation to the total was higher than the third molars.

The prevalence of supernumerary deciduous and permanent teeth ranges between 0.1 a 0.8%⁸⁻¹¹. In this study, however, after six months of sample searching,

it was possible to gather only 5 teeth with this anomaly. Although some studies^{10,11,13-15} suggest that supernumerary teeth are more common in male upper jaw there was no difference among male and female gender in this experiment. Also, in this sample the fourth molar were more frequent in the lower jaw (60%), in contrast to the literature.

Despite the limitations of the present study, it raises contributions in relation to humans fourth molars morphology and it is going be followed up by a second part study where enamel crystallinity will be analyzed using different techniques such as x-ray diffraction an infrared light absorption.

5. Conclusions

Understanding supernumerary teeth morphology provides useful epidemiological and forensics information. Based on this pilot study discoveries it can be concluded that forth molars typically present one main root canal and that they may be associated to other alterations like hypercementosis. Moreover, it can be verified that the fourth molar has a proportionally bigger enamel volume when compared to third molars.

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