eor.istanbul.edu.tr

Official Publication of Istanbul University Faculty of Dentistry

Eur Oral Res 2018; 52: 75-81

Original research

In-vitro analysis of maxillary first molars morphology using three dimensional Micro-CT imaging: considerations for restorative dentistry

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Purpose

The aim of this study was to determine the differences between the positional relationship of the crown contour and the pulp chamber of left and right maxillary first molars, as well as their morphological characteristics by using micro-CT system with reconstruction from a volumetric rendering software.

Materials and methods

In total, 21 extracted maxillary first molars, including 11 left and 10 right teeth, were used. The positional relationship between the crown contour, pulp chamber and morphology of the teeth were investigated three-dimensionally by means of micro-CT imaging.

Results

Closest distance of mesio-buccal pulp horn to enamel surface in mm was calculated as 2.5±0.20 mm for right and 2.29±0.17 mm for left teeth. This difference was statistically significant ($p=0.017$). The means of closest distance of disto-buccal pulp horn to enamel surface were also significantly different between left and right teeth ($p=0.001$). The mean pulp volumes of right side and left side teeth were, respectively, 32.94±3.19 mm^3 and 33.71±2.82 mm^3 but this difference was not statistically significant.

Conclusion

These results suggest that right and left maxillary first molars should be treated differently during preparation of cavities. Further studies must be done with larger samples as well as for other molar teeth in different populations to reveal the morphology of the molar for further considerations in restorative dentistry.

Keywords: *Micro CT; maxillary molars; anatomy; pulp dimensions; restorative dentistry*

Introduction

The main aim of restorative treatment is to ensure the integrity of the teeth and their supporting tissues. Successful restorative procedures lies in the understanding the complex anatomy of the teeth (1). Minimal intervention has been proposed as the primary aim of modern caries therapy (2). Medical and dental interventions should be determined by the underlying scientific paradigms that guide the treatment and the progress of the disease (3). Moreover, it is crucial to select the appropriate treatment strategy in order to minimize the risk of creating pulp complications, as it can affect the quantity of caries excavation, risk of pulp injury and exposure, size of cavity preparation, and selection of capping materials (4). Various complications may occur during access cavity preparation or when locating the canal orifices because of the anatomical differences in maxillary molars.

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Received: 15 February 2017
Revised: 7 March 2017
Accepted: 20 March 2017

DOI: 10.26650/eor.2018.448

How to cite: *Baltacıoğlu İH, Demirel G, Kolsuz ME, Orhan K. In-vitro analysis of maxillary first molars morphology using three dimensional Micro-CT imaging: considerations for restorative dentistry. Eur Oral Res 2018; 52(2): 75-81.*

As maxillary molars usually represent complex anatomy and canal morphology, some studies assessed their anatomical characteristics to contribute to the treatment strategies (5, 6). These teeth may exhibit some anatomic variations and can be challenging cases while performing restorative treatment (7). Previous studies also indicated that access cavity preparation is performed subjectively, which mostly depends on the clinician's tactile perception and knowledge of dental anatomy (1, 4). Two-dimensional methods used for studying morphology of dental tissues are being replaced by three-dimensional ones. The conventional three-dimensional data is obtained by the *in vitro* reconstruction of the images of sample sections under light microscopy (8-10). Micro-CT is an innovative technique that provides three-dimensional data of the teeth, as it can produce this information without destruction of the dental tissue specimen (11). There is a lack of information concerning teeth morphology and pulp orifices in maxillary molar teeth in the literature (12, 13).

The present study therefore aims to evaluate the positional relationship between the crown contour and the pulp chamber as well as morphological characteristics of maxillary first molars using micro-CT system. The null hypothesis tested in this study is that the anatomical and morphological characteristics of right and left maxillary molars do not differ in any of the micro-CT based three dimensional measurements.

Materials and methods

Study sample

Based on the literature, (14-18) a power analysis (Power and Precision software, Biostat, Englewood, NJ, USA) was conducted to determine the sample size. At least 20 teeth at a power of 0.8 (alpha=0.05) was indicated. Thus, this study was conducted using 21 teeth (11 from left, 10 from right) of subjects aged between 20-30 years (mean age for left: 26 and for right: 25). The teeth used in this study had been extracted from the patients who had periodontal problems without carious lesions. Ethics committee approval and written consents from the patients were obtained before the study.

Micro-CT evaluation

A desktop, Micro-CT system in high resolution (Skyscan 1174, Skyscan, Kontich, Belgium) was used to scan the specimen. Before scanning, teeth were rinsed and stored in 0.9% saline solution within a tube. The teeth were placed in upright position on the scanning platform, to which the resorbed roots were fixed with wax. The teeth were scanned at 50 kvp, 100 mA beam current, 0.5 mm Al filter, 18.5 µm pixel size, rotation at 0.5 step, three frame averaging. Furthermore, after scanning of a tooth, in order to minimize ring artifacts, air calibration of the detector was carried out prior to each scan. A ring artifact correction of 0 and beam hardening correction of 40% were applied. Each sample was rotated 360° within an integration time of 5 min. Mean time of scanning was around 2 hrs.

Micro-CT image reconstructions

Reconstructions were performed using NRecon software (v 1.6.7.2, Skyscan, Kontich, Belgium), by means of Feldkamp *et al.* modified algorithm, obtained using a three-dimensional density function based on a series of two-dimensional projections. The NRecon software, by using this algorithm, created axial two-dimensional images. Other settings included beam-hardening correction and input of optimal contrast limits (0–0.0005) were set prior to teeth reconstructions. Contrast limits were applied according to the manufacturer's instructions. To obtain density scale of zero origin, the lowest limit was set to zero. The top of the brightness spectrum was the maximum limit, representing the highest density value. The image data set was approximately 900 axial tomographic slices, each measuring 1024x1024 pixels with a sixteen bit gray level (Figure 1). A 21.3-inch flat-panel color-active matrix TFT medical display (NEC MultiSync MD215MG, Muenchen, Germany) with a resolution of 2048 × 2560 at 75 Hz and 0.17-mm dot pitch operated at 11.9 bits was used to perform all reconstructions and measurements (Figure 1).

Volumetric rendering software analysis

After obtaining the axial images from Micro-CT scanning, the original grayscale images were processed with a Gaussian low-pass filter in order to reduce noise, and then for subtraction of teeth and pulp, an automatic segmentation threshold was used with CTAn (ver. 1.16.1.0, SkyScan, Kontich, Belgium). The images were rendered, and sagittal, axial, and coronal slices and the 3D models were reconstructed (Figure 2). The crown contour, pulp orifices and their positional relationships could be observed three-dimensionally by making the enamel and dentin translucent.

Three-dimensional measurements

The topographic relationship between the crown contour and the pulp orifices were measured. The crown volume/pulp volume and crown/pulp (ratio) was also calculated with the help of the software CTAn in which the user can designate

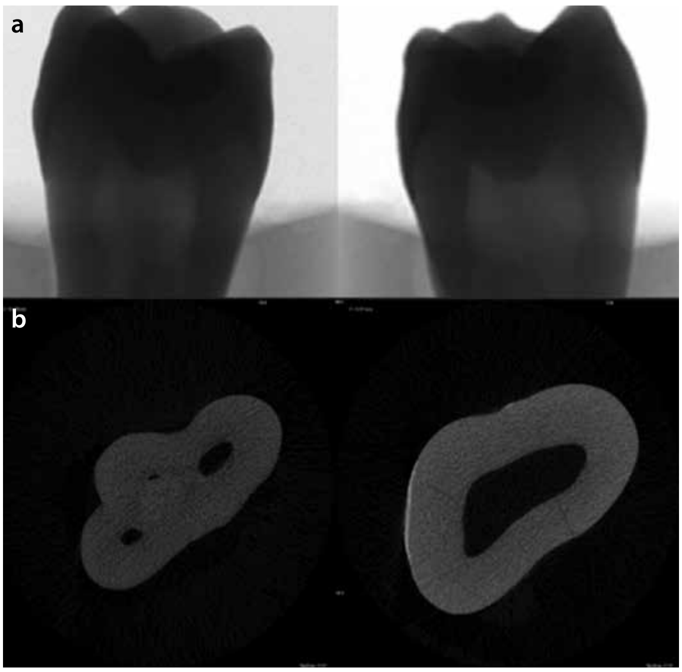

Figure 1. a, b. *(a) 3D scout and (b) axial micro-ct image showing one of the investigated tooth.*

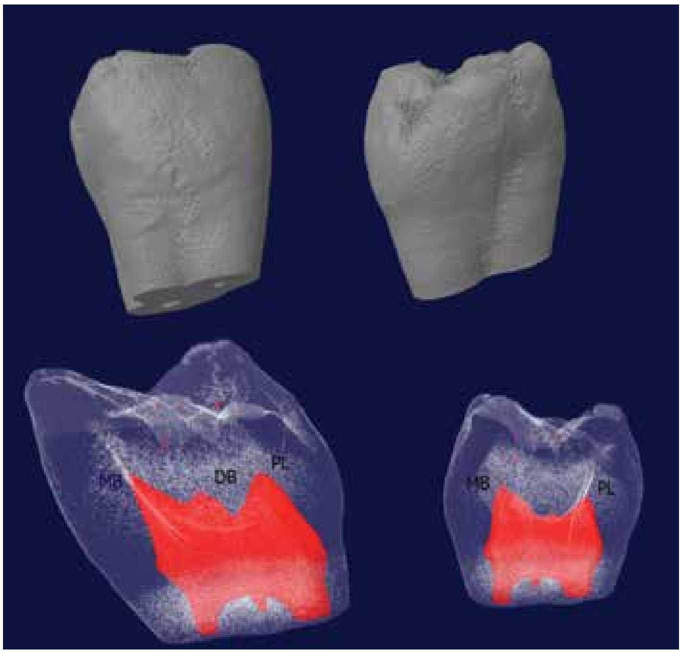

Figure 2. *Three representations of micro-CT image; showing the pulp chamber with hints extending to surface. Note the mesiobuccal horn protuberance.*

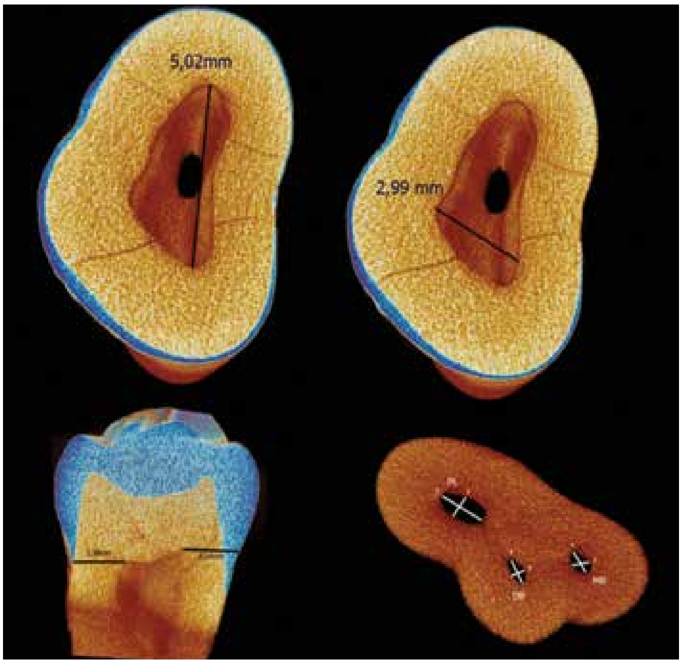

Figure 3. *The color cited representations of micro-CT images with measurements of the investigated parameters.*

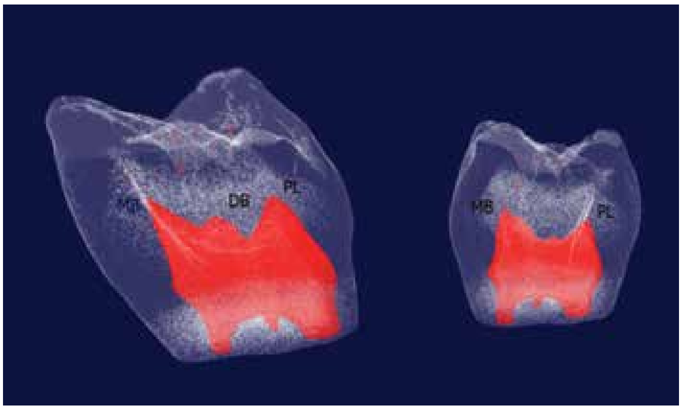

Figure 4. *3D images of the teeth and reflection of pulp and horns.*

the desired volume from the given three-dimentional structure. Also, the observer could remove the unwanted voxels before calculating the final volumes by adjusting brightness and opacity values (Figure 2). Mesial canal orifice length and width (mm), distal canal orifice length and width (mm), palatinal canal orifice length and width (mm), the closest distance from pulp chamber to mesial enamel surface (mm), the closest distance from pulp chamber to distal enamel surface (mm), max. pulp Chamber length and width (mm), closest distance of mesio-buccal pulp horn to enamel surface (mm), the closest distance of disto-buccal pulp horn to enamel surface (mm) (Figure 3, 4). All reconstructions and measurements images were done twice by a dentomaxillofacial radiologist with 18 year of experience (KO) and a restorative dentist with 10 years of experience (IHB) with Micro-CT own CTAn software. Software allows the operator to measure the distances, areas, and volume in life size without dependent of the operator skills. All measurements were done twice by the same observers. To detect intra-observer variability, observers performed their observations twice with an interval of 2 weeks.

Examiner reliability and statistical analysis

The Statistical Package for the Social Sciences 17.0.1 software (SPSS Inc.; Chicago, IL, USA) was used for statistical analyses. Intra- and inter-examiner validations were measured. To assess intra-observer reliability, the Wilcoxon matched-pairs signed rank test was used for repeated measurements. The inter-observer reliability was determined by the intraclass correlation coefficient (ICC) and the coefficient of variation (CV) [CV=(standard deviation/mean)x100%]. Values for the ICC range from 0 to 1. ICC values greater than 0.75 showed good reliability, and the low CV demonstrated the precision error as an indicator for reproducibility (19). Differences in side, dentin thickness and pulp volumes were evaluated using chi-square and paired t-tests. Confidence interval was set to 95% and p-values less than 0.05 were considered as statistically significant.

Results

Intra-observer consistency

Repeated CBCT evaluation and measurements indicated no significant intra-observer difference for both observers. Overall intra-observer consistency for observer 1 was rated at 92.2% and 96.4%, while the consistency for observer 2 was found 91.8% and 94.4% between the two evaluations and measurements, respectively. All measurements were found to be highly reproducible for both observers and no significant difference was obtained from two measurements of the observers.

Inter-observer consistency

The ICCs between Observer 1 and Observer 2 ranged from 0.940 to 0.992. There was a high inter-observer agreement, while a high ICC and low CV demonstrated that the procedure was standardized between the evaluations and mea-

Table 1. Results of statistical analyses stratified by study variables

Study Variables		Group							Mann Whitney U Test	
		n	Mean	Median	Min	Max	SD	Mean Rank	z	p
Pulp Volume (mm³)	Right	10	32.94	32.91	27.84	38.65	3.19	10		
	Left	11	33.71	34.48	28.78	37.5	2.82	11.91	-0.704	0.481
	Total	21	33.35	33.48	27.84	38.65	2.95			
Crown Volume (mm³)	Right	10	130.9	130.5	124	138	4.7	9.05		
	Left	11	133.82	132	128	141	4.4	12.77	-1.381	0.167
	Total	21	132.43	132	124	141	4.68			
Crown/Pulp (Ratio)	Right	10	3.64	3.95	0	4.6	1.31	11.2		
	Left	11	3.99	3.83	3.62	4.45	0.32	10.82	-0.141	0.888
	Total	21	3.82	3.92	0	4.6	0.93			
Mesial Canal Orifice length (x) (mm)	Right	10	1.14	1.13	1	1.29	0.08	9.6		
	Left	11	1.16	1.15	1.08	1.29	0.06	12.27	-0.992	0.321
	Total	21	1.15	1.15	1	1.29	0.07			
Mesial Canal Orifice Width (y) (mm)	Right	10	0.74	0.75	0.6	0.9	0.09	13.45		
	Left	11	0.66	0.66	0.5	0.82	0.09	8.77	-1.73	0.084
	Total	21	0.7	0.7	0.5	0.9	0.1			
Distal Canal Orifice Length (x) (mm)	Right	10	1.49	1.45	1.1	1.8	0.21	10.55		
	Left	11	1.51	1.52	1.2	1.8	0.2	11.41	-0.318	0.751
	Total	21	1.5	1.5	1.1	1.8	0.2			
Distal Canal Orifice Width (y) (mm)	Right	10	0.71	0.69	0.54	1.1	0.16	11.3		
	Left	11	0.67	0.68	0.5	0.8	0.1	10.73	-0.213	0.832
	Total	21	0.69	0.68	0.5	1.1	0.13			
Palatinal Canal Orifice Length (x) (mm)	Right	10	1.7	1.7	1.52	1.8	0.08	5.9		
	Left	11	1.87	1.9	1.79	1.9	0.05	15.64	-3.672	0.120
	Total	21	1.79	1.8	1.52	1.9	0.11			
Palatinal Canal Orifice Width (y) (mm)	Right	10	1.16	1.12	1	1.6	0.17	11.1		
	Left	11	1.13	1.1	0.98	1.2	0.07	10.91	-0.073	0.942
	Total	21	1.15	1.12	0.98	1.6	0.13			
Closest distance from pulp chamber to mesial enamel surface (mm)	Right	10	2.03	2	1.8	2.4	0.18	12.65		
	Left	11	1.91	1.92	1.5	2.2	0.19	9.5	-1.167	0.243
	Total	21	1.97	1.96	1.5	2.4	0.19			
Closest distance from pulp chamber to distal enamel surface (mm)	Right	10	1.91	1.94	1.72	2.2	0.15	11.65		
	Left	11	1.85	1.86	1.4	2	0.17	10.41	-0.464	0.643
	Total	21	1.88	1.9	1.4	2.2	0.16			
Maximum Pulp Chamber length (mm)	Right	10	5.09	5.1	4.8	5.4	0.21	13.45		
	Left	11	4.74	4.6	4.2	5.6	0.49	8.77	-1.731	0.083
	Total	21	4.91	5	4.2	5.6	0.42			

***Table 1.** Results of statistical analyses stratified by study variables (continued)*

Study Variables		**Group**							**Mann Whitney U Test**	
		n	**Mean**	**Median**	**Min**	**Max**	**SD**	**Mean Rank**	**z**	**p**
Maximum Pulp Chamber width (mm)	Right	10	2.94	3.05	2.5	3.6	0.37	8.5		
	Left	11	3.33	3.1	2.7	4.1	0.48	13.27	-1.777	0.076
	Total	21	3.14	3.1	2.5	4.1	0.47			
Closest distance of mesio-buccal Pulp horn to enamel surface (mm)	Right	10	2.5	2.51	2.1	2.9	0.2	14.4		
	Left	11	2.29	2.29	1.9	2.56	0.17	7.91	-2.396	0.017
	Total	21	2.39	2.4	1.9	2.9	0.21			
Closest distance of disto-buccal Pulp horn to enamel surface (mm)	Right	10	3.62	3.61	3.52	3.78	0.08	15.8		
	Left	11	3.41	3.41	3.24	3.58	0.12	6.64	-3.387	0.001
	Total	21	3.51	3.54	3.24	3.78	0.14			
Closest distance of palatal Pulp horn to enamel surface (mm)	Right	10	3.46	3.54	3.50	3.68	0.06	14.2		
	Left	11	3.38	3.34	3.26	3.48	0.10	7.82	-2.986	0.001
	Total	21	3.42	3.44	3.34	3.52	0.08			

surements of the observers. No statistical differences were found among observer's evaluations and measurements. The means of all observer's evaluations and measurements were therefore calculated for further analysis.

Repeated measurements of images showed no significant intra-observer difference. Intra-observer consistency was 92.2% between two examinations, 96.4% between measurements.

Observation of the pulp chamber showed a clear morphological image of the mesiobuccal, distobuccal, mesiopalatinal and distopalatinal pulp horns. Moreover, , a pulp horn corresponding to Carabelli's cusp was seen under the mesiopalatinal cusp (Figure 2). The mesiobuccal pulp horn projected the most, followed by the distobuccal, the palatinal pulp horns (Table 1) (Figure 2, 4). The pulp horn of the mesiobuccal and distobuccal cusp showed protrusion to the crown.

Table 1 shows the results of the evaluations and measurements. There were no statistical significant difference between right and left maxillary teeth canal orifices' length and width. There were also no significant difference in terms of closest distance from pulp chamber to mesial enamel surface (mm) and closest distance from pulp chamber to distal enamel surface (mm) of the maxillary molar teeth.

However, significant difference was found in terms of closest distance of mesio-buccal pulp horn to enamel surface (mm). Mean distance was 2.5±0.20 mm for right; and 2.29±0.17 mm for left teeth ($p<0.05$). Similarly, closest distance of disto-buccal pulp horn to enamel surface (mm) was also significant between left and right teeth ($p<0.05$).

Table 1 also shows the crown, pulp volumes and crown/pulp ratio. There was no statistical significance between pulp volume ratios of pulp chamber and gender. This results indicated that the volume ratio of the pulp chamber to the total crown was approximately same for both sides.

Discussion

Knowledge of the structures of teeth and their relationships to each other contributes to the success of treatment, especially when treating dental caries. Before tooth preparation localization of the caries usually diagnosed radiographically but a 2-dimensional image may not always be accurate. Generally, conventional clinical radiography is used to examine the pulpal anatomy; however, this method only produces a 2D record rather than providing more realistic 3D information (20).

When considering restorative procedures on vital teeth; 3D information of the internal structures is crucial not only for having a proper seal of the remaining tissue under the restoration, but also affect the type of the restoration that will be applied to the teeth (21). The findings of this study suggested that right and left maxillary first molars can differ in terms of internal anatomical appearance. Hence, during tooth preparation in maxillary first molars, care must be taken before any restoration procedure. Moreover, based on the limited findings of the study for this particular population; mesio-buccal pulp horn in maxillary molars are more prominent than the other pulp horns, thus they are more likely to get exposed during tooth preparation.

Different restorative materials require varied thickness to provide resistance of the restorations. Conventional preparations require specific wall forms, depths, and marginal forms because of the properties of the restorative (22). Adequate thickness for amalgam restorations is 1.5–2 mm in occlusal surface and 0.75 mm in axial areas (23), depending on the region, cast metal restorations requires 1 to 2 mm and ceramics 2 mm thickness (24). In addition, these restorative materials require conventional tooth preparation. The use of adhesive restorations, primarily composites and glass ionomers, has allowed a reduced degree of precision of tooth preparations (25). Although some author suggest that composite materials dimensional needs depends on the occlusal wear potential of the restored area, it is generally accepted that, in areas of occlusal loading, minimal thickness of resin composite restorations should be 1.5 to 2 mm (26).

The management of dental caries has evolved from G.V. Black's "extension for prevention" to "minimally invasive", because of advance in adhesive dentistry and restorative materials. Therefore, clinicians should prefer minimal tooth preparation with modified cavity designs and use adhesive dental materials (27). In the present study, it can be suggested that especially in maxillary first molars, adhesive restoration techniques should be preferred to conventional restorations. On the other hand, even when adhesive restoration techniques are used, minimal dentin thickness must be around 0.5 mm with capping or 2 mm without capping (28-30).

Over preparation may perforate pulp chamber or can cause reversible or irreversible pulpitis. It must be kept in mind that capping procedures may be necessary when remaining dentin thickness gets reduced. Hence, future studies must be done regarding modified techniques with newly manufactured materials.

Clinical knowledge on the anatomy of crown-root pulp structure is the key for successful endodontic treatment. Due to the complex root canal system of maxillary first molars, various errors could occur during access cavity preparation or when locating the canal orifices. Having three dimensional information on dental anatomy would definitely help clinicians in the preparation of access cavity. Also, 3-D measurements of pulp chamber, canal anatomy, root orifices will enhance their success in root canal treatment (1).

Conventional methods used for morphological studies on internal anatomy of teeth are destructive *in vitro* methods that generally result in irreversible changes to the specimen (15). As a non-destructive analysis technique, Micro-CT imaging provides objective data. Specimens can be evaluated both quantitatively and qualitatively. In addition, the volumes can be calculated and it is possible to pinpoint specific details with visual image analysis. Filling materials, voids, and tooth structures can be distinguished with high accuracy and spatial resolution (31). With proper lighting, color and texture use during rendering of the image, micro-CT is able to provide a better understanding of internal anatomy of the teeth that could be examined from different angles (14). For the reference, the internal crown anatomy of the teeth investigations especially with and without different restoration materials must be performed using this device.

The study had several limitations. Most importantly, its sample size can be considered low, even though the power analysis indicated that at least 20 teeth should be included in the study for the detection of differences. Micro-CT analysis is an expensive technique; therefore, we had to limit our sample size. The second limitation was the possible effects of sexual dimorphism which were not taken into account due to small sample size. Further studies with larger samples should focus on possible age-, gender- and population-related differences. Also, volumetric and morphologic changes of pulp chamber related to age, chronic caries lesions, and formation of tertiary or reparative dentin, calcifications and other factors were not considered in the present study, which acts as the third limitation factor.

Conclusion

Within the limits of this *in vitro* study, it can be suggested that right and left maxillary first molars should be treated differently during preparation of cavities. Further studies must be done with larger samples, as well as on other molar teeth, in different populations to reveal the morphology of the molars for considerations in restorative dentistry. Development of non-destructive analysis techniques such as micro-CT is of utmost importance to provide clinicians with accurate three dimensional information.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Ankara University Faculty of Dentistry (02.02.2017, 36290600/07).

Informed Consent: Written and verbal informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: İHB and KO designed the study. GD and MEK generated and gathered the data. İHB and KO analyzed the data and wrote the majority of the original draft. GD and MEK participated in writing the paper. All authors approved the final version of the paper.

Conflict of Interest: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Türkçe öz: *Üst birinci büyük azı dişlerinin morfolojisinin üç boyutlu mikro-BT görüntülemesi ile in-vitro analizi: restoratif diş hekimliği için değerlendirmeler. Amaç: Bu çalışmanın amacı üst sağ ve sol birinci büyük azı dişlerinin kron konturu ve pulpa odası arasındaki konumsal ilişkinin farklılıklarını ve morfolojik özelliklerini mikro-bilgisayarlı tomografi (BT) sistemi ve hacimsel yeniden yapılandırma programı kullanarak belirlemektir. Gereç ve Yöntem: Bu çalışmada toplam 21 üst büyük azı dişi (11 sol, 10 sağ) kullanılmıştır. Dişlerin; kron konturu, pulpa odası ve morfolojileri arasındaki konumsal ilişki mikro-BT görüntüleme yöntemi ile üç boyutlu olarak incelenmiştir. Bulgular: Dişlerde mezio-bukkal pulpa boynuzu ile mine yüzeyi arasındaki en yakın mesafe sağ bölge için 2,5±0,20 mm, sol bölge için 2,29±0,17 mm olarak ölçülmüş ve bu farklılığın istatistiksel olarak anlamlı olduğu belirlenmiştir (p=0,017). Disto-bukkal pulpa boynuzu ile mine yüzeyi arasındaki en yakın mesafede de anlamlı farklılık olduğu bulunmuştur (p=0,001). Sağ bölgeden alınan dişlerin ortalama pulpa hacmi (32,94±3,19 mm3) ile sol taraftan alınanların (33,71±2,82 mm3) ortalama pulpa hacimleri arasında anlamı bir fark bulunmamıştır. Sonuç: Bu çalışmanın bulguları kavite preparasyonu sırasında sağ ve sol dişlerdeki farklılıklara dikkat edilmesi gerektiğini göstermektedir. Diğer azı dişlerini de içeren, farklı toplumları inceleyen ve daha geniş örneklem hacmine sahip olan çalışmaların yapılması azı dişlerinin morfolojik özelliklerini ortaya çıkartarak restoratif tedavi alanına katkıda bulunacaktır. Anahtar kelimeler: Mikro BT, üst büyük azı dişleri; anatomi; pulpa boyutları; restoratif diş hekimliği*

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