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Analysis of pulp chamber of primary maxillary second molars using 3D micro-CT system: an *in vitro* study

ABSTRACT

Aim *The aim of this study was to determine the positional relationship between the crown contour and the pulp chamber as well as the morphological characteristics of the pulp chamber using micro-CT in order to plan, in restorations of deciduous maxillary second molars, reconstructions with a volumetric rendering programme.*

Materials and methods *Study Design: In total 16 deciduous maxillary second molar teeth (8 from boys, 8 from girls) were used. The positional relationship between crown contour and pulp chamber was three-dimensionally observed by micro-CT). Differences in sex, dentin thickness and pulp volumes were evaluated using chi-square and paired t-tests. Differences were considered significant when $P < 0.05$.*

Results *Dentin thickness was found to be 2.8 mm ± 0.2, mesiobuccally 3.15 mm ± 0.2 distobuccally 3.8 ± 0.3, which was statistically significant ($p \leq 0.05$). The pulp volume for boys was 77 mm^3 ± 4, for girls 64 mm^3 ± 5, with a statistical significance ($p \leq 0.05$).*

Conclusions *General differences could play a role when planning a treatment for a child; however for both genders it should be noted that mesiobuccal pulp horn is most likely to get exposed during cavity preparation.*

Keywords *Crown contour; Micro-CT analysis; Primary second molars; Pulp chamber morphology.*

Introduction

In order to understand why primary teeth generally need pulpal treatment, their anatomy should be examined [Lewis and Law, 1973]. Enamel and dentin thickness in primary teeth is approximately the half of permanent teeth and the coronal pulp volume-to-crown volume ratio is larger in primary teeth [Lewis and Law, 1973; Mathewson and Primosch, 1995; Johnsen, 2002]. It is also known that dentin of primary teeth is less mineralised than in permanent ones [Johnsen, 2002]. Owing to these anatomical differences, caries easily reaches the pulp [Lewis and Law, 1973; Mathewson and Primosch, 1995; Johnsen, 2002].

The primary objective of pulp therapy is to maintain the integrity and health of the teeth and their supporting tissues [AAPD, 2009]. Pulp therapy requires periodic clinical and radiographic assessment of the treated tooth and the supporting structures [AAPD, 2009]. A radiograph of a primary tooth pulpectomy should be obtained immediately following the procedure to assess the quality of the filling and to help determine the tooth's prognosis. Radiographic evaluation of primary teeth pulpotomies should occur at least annually because the success rate of pulpotomies decreases over time [Holan et al., 2005]. In addition to this, radiographic assesment is an another important key factor in successful treatment: the pulp cavity should be visualised during treatment since variations in size and morphology of both the pulp chamber and the root canals occur in primary teeth [Zoremchhingi et al., 2005].

Two-dimensional methods for studying morphology of dental tissues are being replaced by three-dimensional ones. The conventional three-dimensional data are obtained *in vitro* reconstructing the images of sections of samples under light microscopy [Kimura et al., 1977; Lyroudia et al., 2000; Lyroudia et al., 2002].

Microcomputed tomography is an innovative technique for three-dimensional studies that offers the preservation of specimens [Schwass et al., 2009].

In the literature there are limited investigations on the relation between pulp chamber and primary molar sdimensions [Amano et al., 2006]. Therefore, the purpose of this study was to determine the positional relationship between the crown contour and the pulp chamber as well as the morphological characteristics of the pulp chamber using micro-CT, in order to plan, in restorations of deciduous maxillary second molars, reconstructions with a volumetric rendering programme and compare the differences in morphology between genders.

Materials and methods

Based on the literature [Mikrogeorgis et al., 1999; Amano et al., 2006; Amagetsu et al., 2010; Markvart et al., 2012], a power analysis (Power and Precision software, Biostat, Englewood, NJ, USA) was conducted to determine the sample size, which indicated that differences could be detected with at least 16 teeth at a power of 0.8 (alpha = 0.05). Thus, this study was conducted using 16 teeth (8 from girls, 8 from boys) of subjects aged between 10-12 years (mean age for girls: 11.06 and for boys: 11.18) (Table 1). The teeth used in this study were extracted from patients in mixed dentition stages due to physiological resorption process that had no carious lesions. Written consents from the parents were obtained before the study. All teeth were numbered randomly and the observer was blinded and was not aware if the teeth were from a boy or a girl.

Gender	Min. age	Max. age	Mean and SD
Boys	10	12	11.18±0.70
Girls	10	12	11.06±0.73

TABLE 1 Average age of boys and girls.

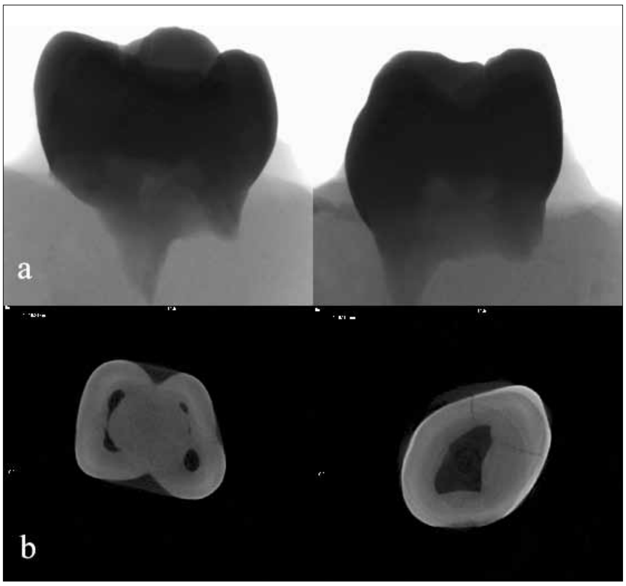

FIG. 1 Figure showing (a) scout Micro-Ct images, (b) axial images of a deciduous second molar reconstructed by Nrecon software.

Micro-CT evaluation

A high resolution, desktop, Micro-CT system (Skyscan 1174, Skyscan, Kontich, Belgium) was used to scan the specimen. Before scanning, the teeth were rinsed and stored in saline solution (0.9%) 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. Futhermore, after scanning of a tooth, in order to minimise 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 analyses

Reconstructions were performed using NRecon software (v 1.6.7.2, Skyscan, Kontich, Belgium) by means of a modified algorithm described by Feldkamp et al. [1989] which was obtained using a three-dimensional density function based on a series of two-dimensional projections. The NRecon software which has this algoratim was used to create axial two-dimensional images. Other settings included beam-hardening correction, as already described above, and input of optimal contrast limits (0–0.0005) were set prior reconstruction of the teeth. Contrast limits were applied following SkyScan instructions. The lowest limit was zero so that the density scale had zero origin. The maximum limit was at the top of the brightness spectrum, representing the highest density value. The image data set was approximately 900 axial tomographic slices, each measuring 102x1024 pixels with a sixteen bit gray level (Fig. 1). All images were reconstructed on a 21.3 inch flat-panel color active matrix TFT medical display (Nio Color 3MP, Barco, France) with a resolution of 76 Hz, 0.2115 mm pitch at 30 bit.

After reconstruction, CTAn scan (v 1.12.9, Skyscan, Kontich, Belgium), was used in order to make objective evaluations on the topographic relationship between the crown contour and the pulp chamber.

Volumetric rendering software analysis

Following axial images that were obtained from Micro-CT, were exported in DICOM file format with a 1024×1024 matrix and imported to Invivo software (v 5.1.2., Anatomage San Jose, CA). 3D surface representations were prepared from DICOM images (Fig. 2). These images were rendered into volumetric images, and the reconstructed sagittal, axial, and coronal slices and the 3D models were obtained. By making the enamel and dentin translucent, the positional relationship between the crown contour and the pulp chamber was three-dimensionally observed (Fig. 3).

Only the pulp volume of each teeth was calculated again with this software. The software itself allows the user to “sculpt out” the desired volume from the three-dimensional structure, and, by adjusting brightness and opacity values, one can remove the unwanted voxels before calculating the final pulp volume.

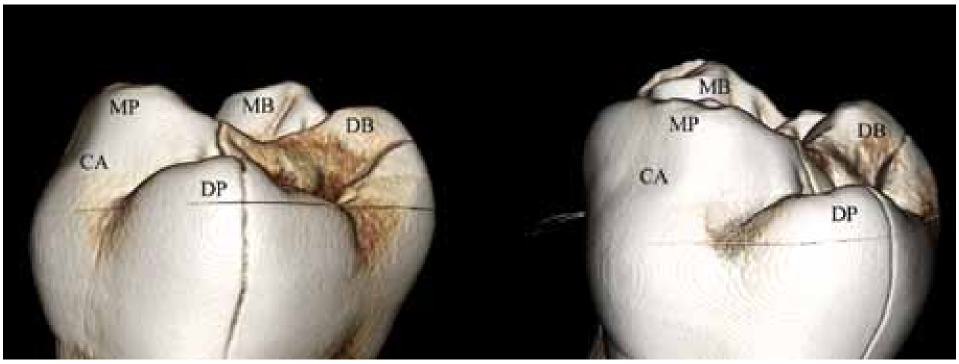

FIG. 2 3D surface representations of crown morphology of maxillary second deciduous molar. DP: distopalatinal cusp; DB: distobuccal cusp; MP:mesiopalatinal cusp; MB: mesiobuccal cusp and CA: Carabelli's cusp.

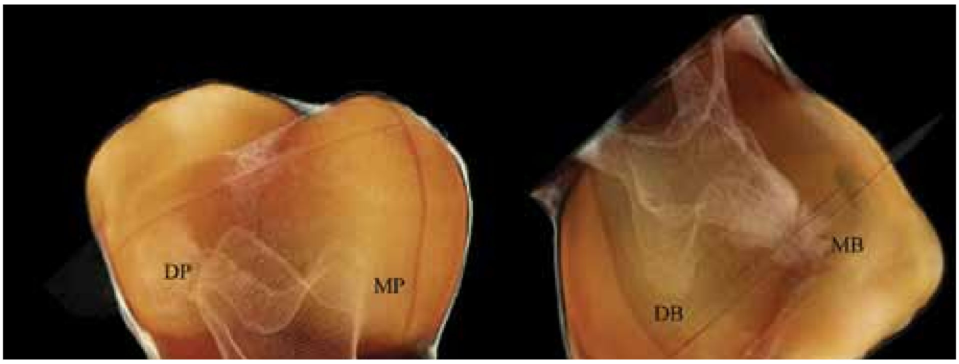

FIG. 3 3D rendered image of pulp chamber morphology of maxillary second deciduous molar. DP: distopalatinal horn; DB: distobuccal horn; MP:mesiopalatinal horn; MB: mesiobuccal horn.

FIG. 4 The dentin thickness measurement from each pulp horn to enamel-dentin border.

The volumes of the total crown and pulp chamber were each calculated using the same software. The pulp chamber-to-total crown volume ratio was also obtained. Furthermore, to understand the dentin width at the pulp horn, sagittal images of the teeth were used to measure the dentin thickness between each pulp horn and the enamel-dentin interface (Fig. 4). All reconstructions and measurements images were done twice by a dentomaxillofacial radiologist with 12 year of experience (KO). All measurements were done twice by the same observer, and the mean values of these measurements were included in the statistical analysis. The observers also performed the study twice with an interval of 2 weeks to detect intra-observer variability.

Examiner reliability and statistical analysis

Statistical analyses were carried out using the SPSS 17.0.1 software (SPSS, Chicago, IL, USA). Intra-examiner validation measures were conducted. To assess intra-observer reliability, the Wilcoxon matched-pairs signed rank test was used for repeated measurements. Differences in sex, dentin thickness and pulp volumes were evaluated using chi-square and paired t-tests. Differences were considered significant when $P < 0.05$.

Results

Repeated measurements of images showed no significant intra-observer difference ($p > 0.05$). Intra-observer consistency was 95.4% between two examinations, 98.6% between measurements.

Observation of the pulp chamber revealed a clear morphological image of the mesiobuccal, distobuccal, mesiopalatinal and distopalatinal pulp horns. Furthermore, under the mesiopalatinal cusp, a pulp horn corresponding to the Carabelli's cusp was seen (Fig. 2). The mesiobuccal pulp horn was the most projecting one, followed by the distobuccal, the mesiopalatinal and the distopalatinal pulp horns (Fig. 4). In relationship to the crown contour and the pulp chamber, the pulp horn of the mesiobuccal and distobuccal cusp showed protrusion to the crown. The pulp was shifted mesially in 81.25% (n=13) of teeth. However, it should be noted that it was protruded more mesiobuccally than distobuccally.

The pulp volume for boys was 77 mm 3±4 and for girls it was 64 mm3±5 with a statistical significance ($p<0.05$) (Table 2). The pulp chamber-to-total crown volume ratio was also calculated by dividing the total crown volume to pulp chamber volume and resulted in 7.2±0.4% and 6.8±0.4% for boys and girls, respectively. There was no statistical significance between gender and pulp volume ratios of pulp chamber. This result indicated that the volume ratio of the pulp chamber to the total crown was approximately the same for both genders ($p>0.05$) (Table 2).

Minimum dentin thickness was measured from all pulp horns. Mean dentin thickness was found to be 2.8mm±0.2 mesiobuccally; 3.15mm±0.2 distobuccally; 3.8 ±0.3 mesiopalatinal and 3.4 ±0.2 distopalantinal (Fig, 4, 5). The buccal horns were thinner than lingual pulp horns which was statistically significant ($p\leq0.05$). Table 3 shows the minimum dentin thickness according to gender. The thinnest location in both genders was the mesiobuccal pulp horn. Significant differences in the dentin thickness were noticed between the mesiobuccal pulp horn and other regions ($p\leq0.05$) (Table 3).

Boys		
Number of teeth	Pulp volume (mm³)	Volume ratios of pulp chamber
1	81	7.60%
2	73	6.90%
3	75	7.00%
4	75	7.00%
5	81	7.40%
6	75	7.20%
7	78	7.30%
8	78	7.20%
Mean	77mm³*	7.20 %
Girls		
Number of teeth	Pulp volume	Volume ratios of pulp chamber
1	69	7.20%
2	65	6.90%
3	64	6.80%
4	61	6.50%
5	59	6.40%
6	63	6.60%
7	66	7.00%
8	65	7.00%
Mean	64 mm³*	6.80%
p value	<0.05*	>0.05
*Indicates statiscal significance		

TABLE 2 Pulp chamber-to-total crown volume ratio of each teeth according to gender.

Discussion

Knowledge of the internal anatomy of the teeth is a key point for a successful endodontic treatment. Generally conventional clinical radiography is used for determination of the pulp anatomy, however this method only produces a 2D image rather than 3D image of a tooth from radiograph [Swain and Xue, 2009].

The traditional methods used for morphological studies of internal anatomy of teeth are invasive *in vitro* methods that generally produce irreversible changes to the specimen. However with the use of Micro-CT, internal anatomy of teeth can be examined noninvasively and more accurately [Dowker et al., 1997; Bjørndal et al., 1999; Swain and Xue, 2009]. Micro-Ct is used in many investigations in dentistry and is thought to be especially useful in studying the morphology of pulp cavity, since it cannot be directly observed by the naked eye [Dowker et al., 1997; Bjørndal et al., 1999; Amano et al., 2006;

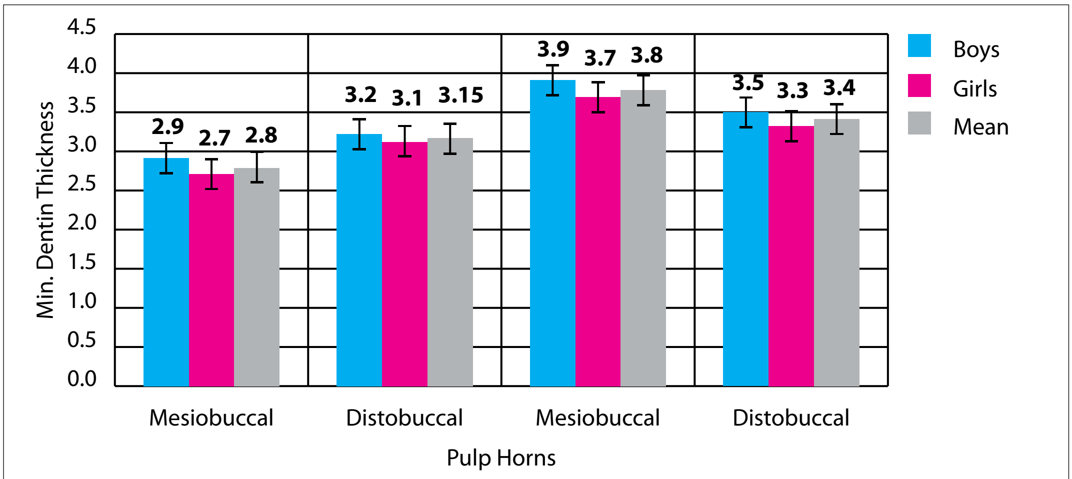

FIG. 5 The mean the thickness of the dentin of both genders according to pulp horns.

Dentin Thickness	Boys			
Number of teeth	Mesiobuccal	Distobuccal	Mesiolingual	Distolingual
1	3.0	3.1	3.7	3.6
2	2.8	3.0	4.0	3.8
3	2.6	3.0	4.0	3.7
4	2.7	3.1	3.5	3.4
5	2.9	3.1	3.6	3.4
6	2.8	3.2	3.8	3.4
7	3.0	3.4	3.8	3.6
8	3.0	3.3	4.0	3.4
Mean (mm)	2.9 [a,b,c]	3.2 [a]	3.9 [b]	3.5 [c]
	Girls			
	Mesiobuccal	Distobuccal	Mesiolingual	Distolingual
1	2.8	2.9	3.5	3.4
2	2.8	3.0	3.8	3.4
3	2.6	2.8	3.8	3.5
4	2.5	2.9	3.4	3.1
5	2.5	2.9	3.8	3.2
6	2.6	3.0	3.5	3.1
7	2.8	3.2	3.7	3.1
8	2.8	3.2	3.9	3.2
Mean (mm)	2.7 [a,b,c]	3.0 [a]	3.7 [b]	3.3 [c]

TABLE 3 Table shows the minimum dentin thickness of each pulp horn according to gender.

Superscript letters indicate statistical significance ($p<0.05$)

Same letters show statistical significance within the same group.

Cheung and Cheung, 2008; Swain and Xue, 2009; Hamba et al., 2012]. It is also known that one of the advantages of the micro-CT is with proper lighting, color and texture use during rendering of the image method provides a better understanding of dental structures, since internal anatomy of the teeth can be examined from different angles [Mikrogeorgis et al., 1999].

Arpana et al. [2010] examined crown and coronal pulp dimensions in carious and non-carious primary maxillary and mandibular second molars using AutoCAD and Dexis analysis of standard bitewing radiographs. The results of the study showed that crown and pulp dimensions were significantly greater in non-carious primary molars. Rhodes et al. [1999] investigated the micro-CT analysis of root canals in mandibular first molar teeth. They concluded that micro-CT is an accurate method for experimental endodontic investigations.

A study using micro-CT for 3D analysis of pulp cavity of molar teeth showed that mineral deposits are more evenly distributed in small pulp chambers compared to large ones. The results of the study showed that 3D surface models analises are made with high precision using micro-CT, as this helps to improve the understanding of tooth morphology [Markvart et al., 2012]. This study is in line with previous ones which indicated that Micro-Ct is a useful tool to understand the morphology of teeth, especially in areas which cannot be directly observed by the naked eye as stated by Amano et al. [2006].

Pulp morphology of maxillary deciduous molars has been studied using traditional methods by Nozaka et al. [1990]. They used sliced specimens and layered them up to obtain a 3D reconstruction to make the analysis, however they did not specify the tooth used in the study.

Amano et al. [2006] investigated the crown contour and pulp chamber morphology of deciduous maxillary second molars in different dentition periods. They concluded that in relationship to the crown contour and the pulp chamber, the pulp horn of the mesiobuccal cusp showed greatest protrusion and swelling of its mesial wall in both the deciduous dentition and the mixed dentition periods. The volume ratio of the pulp chamber to the whole crown in the mixed dentition period showed to be significantly smaller than in the deciduous dentition period.

Agematsu et al. [2010] investigated the changes in pulp cavity volume according to age using micro-CT in human permanent teeth. Their findings showed that volume decrease was slightly higher in females than males with age; also, there was a higher correlation between decrease of volume and aging among females.

Ma et al. [2013] investigated volume measurements of deciduous mandibular first incisors and concluded that the pulp chamber volume decreased and the volume ratio of the pulp chamber with respect to the whole crown significantly reduced with age. Also, the results showed that males had significantly larger tooth crown volumes than females.

In the present study we aimed to investigate gender differences in anatomy of crown contour and the pulp chamber of deciduous maxillary second molars. Keeping in mind that Amano et al. [2006] showed that stage of dentition also plays a role in pulp chamber volume, the teeth used in the present study had been extracted due to resorption of the roots and were close to exfoliation time. By choosing the teeth close to exfoliation time we hoped to achieve more accurate comparisons between the groups, since once a tooth has erupted and starts to occlude, the crown and pulp chamber within start changing their contour and morphology.

The study has some limitations. First of all the sample size is small. Although, the power analysis indicated that at least 16 teeth should be included in the study for the detection of differences, the difficulties to achieve caries-free primary teeth at the time of exfoliation and economical limitations led us to limit the size of the study group.

The second limitation of the study was the side of the teeth. Since the sample size was small, the study group was not divided into left and right teeth, thus any difference regarding the side was not compared.

A larger sample is needed and further studies should be done according to different populations.

The results of the present study showed that the pulp horn of the mesiobuccal and distobuccal cusp showed protrusion to the surface of the crown. However, it should be noted that the mesiobuccal pulpal horn was protured more than the distobuccal. Dentin was significantly thinner mesiobuccally. The pulp chamber volume was greater for boys. The findings of this study suggest that girls have smaller pulp chamber volume than boys in primary dentition, which is in line with the findings of the study conducted in permanent and primary teeth [Arpana et al., 2010] .

These results suggest that in preparation of cavities in maxillary second deciduous molars, care is necessary not to expose of the mesiobuccal pulp horn. Especially, the mesial wall of the mesiobuccal pulp horn and sexual dimorphism should be noted.

Conclusion

Analyzing the tooth by micro-CT allows 3-D visualisation and may be a useful tool to understand the morphology of each tooth. The knowledge of the internal anatomy of the teeth will help the dentist to perform more successful treatments. General differences could play a role when planning a treatment for a child, however for both genders it should be noted that the pulp is shifted to the mesial position, thus the mesiobuccal pulp horn is most likely to get exposed during cavity preparation.

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