Density and diameter of dentinal tubules of first and second primary human molars - comparative scanning electron microscopy study

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The aim of the present survey was to evaluate tubule density and diameter of dentin of first and second primary human molars and compare the two dental categories. These evaluations were done solely at the middle third of the crowns of twenty extracted noncarious teeth separated in two groups (first molars and second molars). The tubule diameters observed were 0.794µm and 1.0µm for first and second molars, respectively (measurements done at 35-65% from the pulp-chamber walls). Regarding tubular density, the measurements indicated 17,997.594 tubules/mm² and 25,211.317 tubules/mm² for first and second molars, respectively. Comparisons between-groups indicated that tubule diameter and density of dentin were higher in second molars, being the difference highly significant (P<0.01).
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INTRODUCTION

A continuous evolution has been observed in the last years not just in techniques, but mainly in restorative materials for the treatment of carious teeth. Among the developed studies with primary teeth, a great part of these is related to the behavior of dentinal tissue when using adhesive systems.[3,7,13-15,19,31,37,39,42,44,49,50]

Bond strength studies on primary and permanent dentin have been demonstrated a variability on the results.[7,13,15,31,50] In agreement with Nor *et al.*[41], in 1996, differences between the primary and permanent dentin could probably be responsible for the different performance of the two dental types in the adhesion tests. In the literature, some peculiarities have been described in relation to the primary dentin.[1,2,23-26,35,36,52]

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The most important dentinal structural characteristic, even in primary teeth as well as in the permanent ones, concerns the tubular characteristics.[47,58,60] According to Torneck[60] the tubular dentinal component is very complex in structure and composition. The knowledge of the dentinal tubular pattern is extremely important to understand the behavior of adhesive systems when applied to it. In the literature, there are several studies establishing correlation between dentinal density and tubular diameter with adhesion values in this tissue.[10,34,45,46,53,54,57]

Noyes[43] in 1912 showed the relationship of density and tubular diameter. This relationship became a great field of investigation. After that, many studies were done.[9,12,16,17,20-22,28,33,40,61] A few of them referred to the density and tubular diameter in dentin of the primary teeth.[11,32,35,51,52]

In 1976, Garberoglio and Brännström[22] demonstrated the variation in diameter and tubular density in different depths of the coronary dentin of permanent teeth. They observed that close to the pulp, the tubular density was 45,000 tubules/mm² and the tubular diameter was 2.5µm. In the medium area of dentin the density was 29,500 tubules/mm² and the diameter 1.2µm, while close to the enamel the number of tubules for millimeter was 20,000 and diameter 0.9µm.

In 1994, Koutsi *et al.*[35] accomplished measurements at the scanning electron microscope of diameter and tubular density of primary molars under four different dentinal depths. For the tubular density they found the following medium values: 17,433 tubules/mm² to superficial dentin, 18,075 tubules/mm² to external dentin, 20,433 tubules/mm² to intermediate dentin and 26,391 tubules/mm² to deep dentin. Concerning the tubular diameter, these authors found the following medium values: 0.96μm to superficial dentin, 1.08μm to external dentin, 1.10μm to intermediate dentin and 1.29μm to deep dentin.

Thus, it can be deduced that more detailed research of the dentinal tubular pattern of primary teeth is necessary. Therefore, the purpose of this scanning electron microscopy study was to determine the diameter and tubular density in the coronary middle third of first and second human primary molars and to observe if there is a different range between the two dental categories.

METHODS AND MATERIALS

Selection of the teeth

For the present study, 20 primary non-carious molars were obtained from children age nine to eleven years old. All teeth were removed for orthodontic reasons with informed consent from parents.

For this study the selected teeth should present from two-thirds to completed root resorption observed by radiographic exam. After the extraction they should not present resorption clinically visible in the internal walls of the pulpal chamber.

After establishing the criteria mentioned above to select the teeth, from eighteen primary first molars and thirteen primary second molars initially obtained, twenty primary molars (seven, first molars and thirteen, second molars) were used in this study.

Immediately after the extraction, teeth were washed with running water and stored in a 10% buffered formalin solution.

Teeth preparation and dentinal reductions

The selected primary molars were divided in two groups in agreement with the dental type, i.e. first and second primary molars. Then dentinal samples were obtained based on the dental type.

The selected teeth were fixed in resin cylinders (20 mm X 15 mm) with the occlusal surface turned to the superior central portion of the apparatus. The fixation was done with self-cured acrylic resin (Classic; São Paulo; Brazil) after the conditioning of occlusal surfaces during 1 min with 35% phosphoric acid gel (3M Co; São Paulo; Brazil).

After that, the primary molar crowns were demarcated in three parts corresponding to the cervical, middle and occlusal coronary thirds. Based on this, the coronary cervical third was separated from the remaining crown by cutting it with a double-faced diamond disk (Horico; Berlin; Germany) under refrigeration.

To obtain the dentinal samples, the dentin was cut with the same diamond disk (in the cervix-occlusal direction) in the middle portion between the dentino-enamel junction and the wall of the pulpal chamber. Each cut corresponded to a face (Figure 1).

Aiming to quantify the distance from the pulpal chamber to the dentinal surfaces in the place of the cuts, a measurement was obtained with a micrometer (Mitutoyo; Tokyo; Japan). The dentinal thickness of the pulp-chamber wall, till the two cut surfaces, as well as until the dentino-enamel junction were registered. The measurements were made in the central portion of each face by a straight line that extended from the wall of the pulp-chamber to the points referred above.

Having the values of the total dentinal thickness and the distance of the dentinal surfaces in relation to pulp-chamber wall, it was determined to what distance were such surfaces in relation to the pulp.

After establishing the measurements, a sequence of cuts was completed to separate the samples of the dentinal surfaces exposed for further analysis of the density and tubular diameter. After that, the dentinal samples of the coronary middle third were separated discarding the occlusal third. After the cuts, eight dentinal samples were obtained by each tooth, making a total of a hundred and sixty samples.

Specimen preparation to scanning electron microscopy (S.E.M.)

The surfaces of the samples to be analyzed were prepared according to the method of smear layer removal advocated by Ruschel *et al.*[48] The application of air-abrasive bicarbonate system plus water jet (Profilax II, Dabi Atlante, São Paulo, Brazil) at a distance of 0.5-1.0cm from the sample, with maximum pressure as indicated by the unit LED, for 60 seconds. Then, the samples underwent ultrasonication treatment (Thorton-T14, Thornton Inpec Eletrônica, São Paulo, Brazil) for 15 minutes.

After smear layer removal, the samples were air dried for a minimum time of 12 hours, in accordance with methodology advocated by Arends *et al.*[4] Each sample was fixed in a specimen mount with the surface to be analyzed turned upward (Sigma Chemical Co, Saint Louis, USA) and properly numbered for subsequent identification.

The samples were vacuum coated (Balzers-SCD 050, Balzers Elaborates, Bonn, Germany), with a 10nm film of gold during 3 minutes (voltage of 460 V and current of 40 mA), for subsequent analysis by S.E.M..

Scanning electron microscopy (S.E.M) and measurement of density and dentinal tubule diameter

For further measurement of dentinal tubular density and diameter, eletromicrographies were obtained at a scanning electron microscope (Stereoscan 200, Cambridge, England), with a voltage of 10Kv. The eletromicrographies were made at the central portion of each sample, with magnification ranging from 2,280x to 2,770x, using for this a Neopan film DX 135 SS (Fuji, Tokyo, Japan).

From 160 dentinal samples obtained in the beginning of this study, eleven were lost during microscopic prepa-

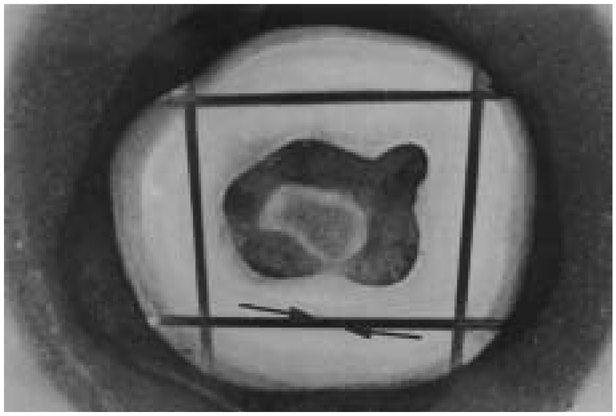

Figure 1. Dentinal surfaces (arrows) to be analyzed after being cut with the diamond disk.

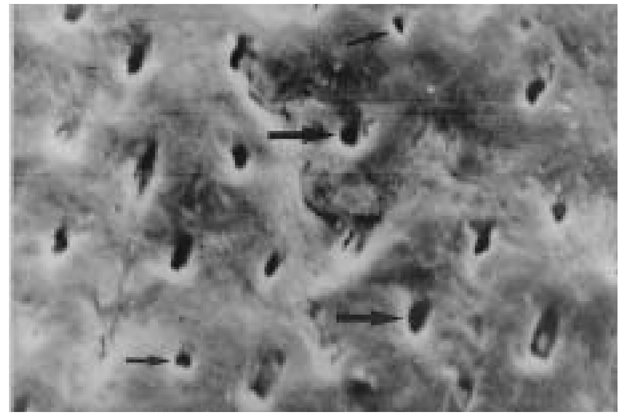

Figure 2. First primary molar dentin eletromicrography showing the largest tubular diameter (bigger arrows) in comparison with the smallest ones (smaller arrows).

ration procedures. From 149 photographed samples, those which did not present the tubules in an appropriate position for the measurement of density and tubular diameter, in other words, tubules cut with a very oblique or longitudinal inclination, were excluded in this phase. Besides, the samples, which were not framed in a distance from 35 to 65% of the pulp, were also discarded. This exclusion was done because the samples with values below 35% or above 65% from the pulp were very few. With that, the measurement of density and tubular diameter was obtained in ninety-six samples (34 were from first molars and 62 from second molars).

From the negatives of eletromicrography films, copies were made in slide films (Elite Chrome, Kodak, New York, USA), for the purpose of doing the measurement through projection.

The measurements were done with the projection of eletromicrographies slides in a white picture (City Quadros Ind. Com. Ltda, Rio de Janeiro, Brazil), using a projection area of 72.5 x 51.5cm. To determine tubule number per square millimeter, all the present tubules in the projected image were marked in the picture with the use of a demarcation pen (Faber-Castell S.A., São Paulo, Brazil). Where tubules intersected the edges of the eletromicrography projected image, only those, which intersected the top and right-hand margins, were included in the total.

To determine tubular diameter, measurements were obtained from two to five tubules of the most central portion of the eletromicrography projection, inside a corresponding area about 18% of the total area projected (33.0 x 20.5 cm). The measurement of tubular diameter was done using a triangular scale ruler (Archimedes 50/1 - Scale 100, Arquimedes Technical Material, Rio de Janeiro, Brazil), where the measurement technique was done as proposed by Arends *et al.*[4] Taking into consideration the measurement of the diameter from the smallest circular dimension of each tubule. This means that the diameter was measured despite the tubule orientation.[4]

With the tubular measures of dentinal samples obtained in centimeters, the values of tubular density were changed to tubules per square millimeter (tubules/mm^2) and the diameters to micrometer (μm).

Statistical analysis

Each sample could present from two to five measurements for the tubular diameter, the mean value and the standard deviation were calculated for this variable in each one of the samples.

In the two dental groups were calculated the mean, standard deviation, minimum value, maximum value, median, first quartile and third quartile for diameter and tubular density.

Aiming to discover if there is a difference between the dental groups in relation to the two analyzed variables, the non-parametric Mann-Whitney test was used, with level of significance of 5%.

RESULTS

Table I shows the mean values of dentinal tubules diameter for the two dental groups. The mean value of tubular diameter was superior in second primary molars (1.0 ± 0.225 μm) in comparison with the first molars (0.794 ± 0.265 μm). This difference was highly significant (p-value=0.0002; Mann-Whitney test).

The values range of tubular diameter for the two dental groups was similar, which was seen by comparing the difference between the maximum and minimum values (1° molar = 0.954 μm; 2° molar = 0.876 μm), the standard deviation and the interquartilical difference (1° molar = 0.440 μm; 2° molar = 0.361 μm). Besides, symmetry in the distribution of values was also verified individually in the groups analyzed by the similarity between the mean value and median (Table I). The value ranges of tubular diameter, in

Table I. Comparison of tubule diameter in coronary dentin between first and second primary molars.

Statistics Descriptive	Diameter (μm) 1st molar	2nd molar
Mean	0.794	1.000
SD	0.265	0.225
Minimum	0.368	0.537
Maximum	1.322	1.413
1st Quartile	0.539	0.825
Median	0.804	0.993
3rd Quartile	0.979	1.186

Note: Mann-Whitney Test; p-value = 0.0002; Highly significant. Values obtained in coronary dentin at 35-65% from the pulp-chamber wall.

Table II. Comparison of tubule density in coronary dentin between first and second primary molars.

Statistic Descriptive	Density (tubules / mm²) 1st molar	2nd molar
Mean	17,997.594	25,211.317
SD	4,744.044	8,111.314
Minimum	8,637.185	11,656.210
Maximum	25,247.156	41,192.728
1st Quartile	14,616.774	19,802.960
Median	17,967.584	25,519.454
3rd Quartile	20,912.61	30,169.02

Note: Mann-Whitney Test; p-value = 0.007; Highly significant. Values obtained in coronary dentin at 35-65% from the pulp-chamber wall.

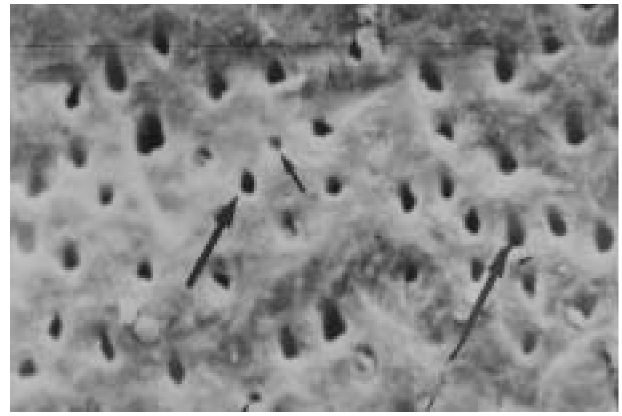

Figure 3. Second primary molar dentin eletromicrography showing the largest tubular diameter (bigger arrows) in comparison with the smallest ones (smaller arrows).

similar analyzed dentinal area, could be observed in Figures 2 and 3.

Concerning tubular density (Table II), the group of second primary molars presented a higher mean value (25,211.317 ± 8,111.314 tubules/mm^2) than first molars (17,997.594 ± 4,744.044 tubules/mm^2), being this difference highly significant (p-value = 0.007; Mann-Whitney test). Such difference in tubular density can be verified comparing eletromicrographies of Figures 2 and 3.

A higher range of tubular density values was obtained in second primary molars group. This was observed through the analysis of the difference between the maximum and minimum value (1° molar = 16,609.971 tubules/mm^2; 2° molar = 29,536.518 tubules/mm^2), of the standard deviation and interquartilical difference (1° molar = 6,295.838 tubules/mm^2; 2° molar = 10,366.040 tubules/mm^2). Furthermore, the tubular density of two dental groups demonstrated symmetrical distribution verified by the proximity of the mean value in relation to the median (Table II).

DISCUSSION

In order to analyze and discuss the values found in this study, some aspects of the sample selection should be discussed.

All selected teeth presented from two thirds to a complete root resorption. With this, dentinal alterations observed by dental aging process, just as dentinal sclerosis [27,47,60], were probably present in both dental groups.

In addition, all selected primary molars were non-carious teeth. Bevelander and Benzer[5] observed that carious lesions lead to a larger formation of sclerotic dentin in primary and permanent teeth. With that, the risks of alterations in the diameter and tubular density would be smaller because non-carious teeth were used in this study.

The samples were prepared very carefully in order not to any alter the tubular diameters before the S.E.M. analysis. To remove the smear layer a mechanical method was developed.[48] The acid etching technique removes the smear layer[41,42] and also the peritubular dentin.[6,18,29,55,56,59,60] Garberoglio and Brännström[22] observed, in a study, that tubular diameters in samples of decalcified dentin were larger than the other samples not conditioned. According to these authors, in the decalcified samples, the removal of peritubular dentin occurred.

In the present study and the literature, referring either to permanent teeth[9,12,17,22,40] or primary teeth,[11,35,51,52] a higher variation in density and tubular diameter was found. In the first primary molars of this study, tubular density varied from 8,637.185 to 25,247.156 tubules/mm^2, while in the second molars it varied between 11,656.210 to 41,192.728 tubules/mm^2. The tubular diameter varied from 0.368 to 1.322μm in the first molars and 0.537 to 1.413μm in the second molars.

Among factors that might have influenced the variation of density and tubular diameter values, it is suggested the use of different origins of dentinal samples. Such samples were obtained from superior and inferior molars from different individuals, as well as different dental surfaces. Torneck[60] reported a higher density and tubular diameter in vestibular and lingual surfaces, when compared with proximal.

Technical factors might also have influenced the mean values of density and tubular diameter of this study. The extent of those factors was difficult to determine. According to Forssell-Ahlberg *et al*.[16] some factors might have influenced the data obtained in the study regarding dentinal tubular density and diameter, such as magnification value used in the eletromicrographies, the tilt of the studied area and difficulties in defining the precise tubular diameter in the pictures.

Comparing the present research to the study done in permanent teeth by Garberoglio and Brännström,[22] it was observed that the density and dentinal tubular diameter in primary molars were smaller to the corresponding values in permanent teeth analyzed by the referred authors. In the middle dentin, Garberoglio and Brännström[22] found the mean value of 1.2μm for the tubular diameter and 29,500 tubules/mm^2 for density. These values were superior to the ones found in the first and second primary molars in this study.

Trying to establish a comparison between primary molars of the present study with their successors teeth, premolars, it is possible to analyze the study of Fosse *et al*.[17] They studied the tubular density of premolars under three different dentinal levels in relation to the pulp. Such authors found a tubular density in the medium portion of dentin from 33,819 to 43,177 tubules/mm^2. With that, the mean values of tubular density found in primary molars were inferior to values reported for premolars by Fosse *et al*.[17]

Establishing comparison between permanent molars with the primary molars in the present survey, the values of the tubular density of third permanent molars obtained by Dourda *et al*.[12] can be used. These authors found a tubular density of 37,000 tubules/mm^2 in the middle dentin. Comparing with the mean value of first primary molars (17,997.594 tubules/mm^2) and second ones (25,211,317 tubules/mm^2), it can be observed that these are less than the permanent molars evaluated by of Dourda *et al*.[12]

According to the literature[12,16,17,22] and the results of the present study, it was verified that density and tubular diameter values of human primary molars were inferior to permanent teeth. Exception should be made to the value of premolar tubular density, in comparison with primary molars, in the study of Kaga *et al*.[32] Such authors found that dentinal tubular density in primary molars was twice that of premolars.

Koutsi *et al*.[35] also reported a study that density values and tubular diameter in primary molars were less to than that reported in permanent teeth for Garberoglio and Brännström.[22] The authors did not describe the reason for such difference. Through a study by scanning electron microscope by Hirayama *et al*.[26] suggested that dentinal tubules in primary teeth presented a smaller diameter because of the largest thickness of the peritubular dentin found in these teeth in comparison with permanent ones. The same authors observed that primary peritubular dentin is two to five times thicker than permanent one.

Koutsi *et al*.[35] studied the permeability, diameter and tubular density in primary molars under different dentinal depths. To compare the results of the present study with these authors, some observations are valid. Firstly, the authors did not specify which types of primary molars were used (first or second molars). Secondly, dentin referred by the authors as intermediate corresponded a 30.1 to 60% distance from pulp, because dentin related to enamel was subdivided in superficial and external. In addition, the primary molars in Koutsi *et al*.[35] study was obtained with children in the same age group as the present study (9 to 11 years old). The primary molars of both investigations can be put in the same period of post-eruption age.

In this research, the values of tubular diameter in the middle third of the crowns of first and second primary molars were respectively 0.794μm and 1.0μm, smaller in the value to intermediate dentin (1.10μm) observed by Koutsi *et al*.[35] Such difference could be explained by the fact that the analyzed area by these authors was coronary cervical dentin, and in the present study it was the coronary middle third.

According to Maroli *et al*.[38] coronary cervical third presents a larger density and tubular diameter that could explain the largest dentinal permeability in the area found by the authors. Koutsi *et al*.[35] also evaluated dentinal permeability in cervical third because they consider it a more permeable area.

Based in the studies above, the smallest values of first and second molars found in this present research in relation to Koutsi *et al*. study[35] could happen due to the fact of the coronary cervical area examined by such authors presented a larger density and tubular diameter, in comparison with coronary middle area analyzed in this present research.

The mean value of tubular density of the present study in first primary molars (17,997.594 tubules/mm^2) was smaller than the one observed by Koutsi in the intermediate dentin (20,433 tubules/mm^2).[35] The second primary molars presented a higher tubular density (25,211.317 tubules/mm^2) than primary molars of the referred study, in spite of the variation in the tubular density of 11,656.210 to 41,192.728 tubules/mm^2 presented in the second molars. Then, the mean values of the tubular density found by Koutsi *et al.*[35] are matched in the variation of values in the second molars of the present research.

The present study separated the primary molars in two different groups (first and second molars). However, more detailed studies are necessary to verify if there are differences in diameter and tubular density of primary molars taking into consideration the following variables: superior and inferior teeth, age group, dental surface analyzed, coronary thirds, coronary and root dentin, dentinal depth, as well as primary teeth from different races and sex.

Clinical Considerations

According to the literature, it can be concluded that dentinal tubular compartment is very complex, exhibiting considerable variations in structure and components. Probably, these differences in dentinal structure are related to different repercussions of clinical point of view.

In 1994, Dourda *et al.*[12] reported that variations in density and dentinal tubular diameter are clinically significant in the permeability of this tissue and in transmission of pain. According to these authors, it is difficult to establish the influence of tubular diameter variation, in different dentinal levels, with hydrodynamic theory of dentinal sensibility proposed by Brännström *et al.*[8] However, it can be expected that pain transmission can suffer variation along the entire dentin.

In the present study, where density and tubular diameter were superior in second molars in relation to first primary molars, others studies would be necessary to verify if such difference would have an influence in terms of different permeability patterns and dentinal sensibility in such teeth.

Sumikawa *et al.*[52] concluded that the primary dentin shows substantial differences in the microstructure compared to the permanent one. Therefore, such differences may be important factors in tooth sensitivity, susceptibility to trauma, and caries progression.

Mjör and Nordahl[40] based in a study about density and dentinal tubules ramifications in human dentin. They suggested the need of more detailed characterization of dentinal substrate in adhesion tests and in studies of dentinal permeability. Suzuki and Finger[53], and Mjör and Nordahl,[40] reported that the variation in adhesion tests, described among different studies, could be attributed to differences of dentinal substrate, more than to the differences among tested products.

In the present research, the smallest density and tubular diameter in first primary molars in relation to the second ones inside of a similar analyzed surface area, conferred to the first ones a more solid dentinal structure, because of smallest tubular pattern. Studies have been demonstrating that an availability of solid dentin is an important factor to obtain better dentinal adhesion.[10,53] Then, it would be interesting to determine if the solid dentinal structure in first primary molars would interfere in adhesion values at this surface.

Trying to discover the interaction between primary dentinal tissue with adhesive systems, as well as if there are differences in adhesion between primary and permanent teeth, some studies have been developed.[3,7,13-15,19,31,37,39,41,42,44,49,50] Fagan *et al.*[15] did not find differences in values of bond strength between primary and permanent teeth. However, other studies as Salama and Tao,[50] Bordin-Aykroyd *et al.*[7], and Jumlongras and White [31], reported smaller values in primary teeth when compared to permanent ones.

According to Nor *et al.*[41], little is known about dentinal tissue of primary teeth, as well as the differences in relation to permanent ones. Probably, according to these authors, differences in chemical composition of dentin[24,30] and micromorphology[35] could be responsible for different results of primary and permanent dentin in adhesion tests.

Sumikawa *et al.*[52] observed that the area of solid dentin that is available in the primary dentin for bonding is significantly reduced, taking into account the reported differences in bond strength compared to permanent dentin.

Besides, the different tubular pattern found in first and second primary molars should be taken into consideration when adhesion test to dentin is evaluated in these teeth, because dentinal tubular pattern has great influence in bonding mechanism of adhesive systems to this tissue.[10,34,46,53,57] Then, a better understanding of primary dentin will improve the adhesive methods, and dental procedures will be more effective.

Concluding the discussion of the present study, there are a few published works about primary teeth, in which some of dentin peculiarities have already been observed. Considering these peculiarities, larger studies are necessary to verify until what extent these differences would determine different clinical conducts from those applied in permanent teeth. More studies about primary and permanent dentinal tissue should look for a higher scientific knowledge to be used in clinical procedures. So they will have the necessary biological data that have been forgotten many times.

CONCLUSIONS

Based on the results of the present study, it is concluded that:

1. The mean values of tubular diameters, in coronary middle third and 35-65% from distance of pulp, were 0.794μm and 1.0μm for first and second molars respectively. The mean values of tubular densities found in first and second molars were 17,997.594 tubules/mm^2 and 25,211.317 tubules/mm^2, respectively,
2. The values of diameter and tubular density in dentinal analyzed area were superior in second primary molars in comparison to first primary molars, the difference was highly significant.

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