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Original

Age-related Changes in Root Dentin
- Measurement of Hypercalcified Root Dentin Using Monochromatic Synchrotron Radiation X-ray Micro-CT-

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Abstract: Polychromatic radiation is a method of measuring errors in hard tissue and is generally used for micro-CT. The study aims to estimate the age by non-destructive observation of hypercalcified dentin using monochromatic radiation x-ray micro-CT (MR-μCT) in synchrotron radiation facility (SPring 8 by RIKEN, Hyogo) eliminating the effects of beam hardening and scattered radiation. We confirmed the mineral density concordance ratio with MR-μCT and CMR. Using MR-μCT, we also investigated the relationship between hypercalcified dentin and age. The mineral density concordance rate in MR-μCT and CMR image was obtained in 44 human teeth by comparing the brightness values. The images of hypercalcified dentin were set for CMR and micro-CT after extracting from MR-μCT and the relationship between age and elapsed time years after root completion was investigated. Results showed a mineral density concordance rate of 90% or more in all samples. Hypercalcification of dentin positively correlated with age and also with elapsed time. However, the increase in values slowed down at over 40 years and after tooth eruption over 31 years. The results suggest that age estimation using hypercalcified dentin is possible through micro-CT.

Key words: Monochromatic synchrotron radiation x-ray μCT, Transparent dentin, Sclerotic dentin, Aging change, Age estimation

Introduction

Various changes in each organ of the human body occur with age. Physiochemical, morphological and histological changes occur with age in soft tissues, such as the pulp, and in hard tissues such as the enamel and dentin. Age-related changes in teeth were reported to be most prominent in dentin[1]. Age-related changes in dentin are characterized by the appearance of transparent layer in sclerotic or root dentin by wear and attrition of the dental crown in accordance with the formation of secondary dentin[2]. Among the changes in dentin, the area of sclerotic dentin appears to increase with age from the root apex toward the tooth crown, thereby it is considered to be a useful tissue for age estimation of the human body[3].

It has been reported that transparency and hardness increased due to the deposition of apatite crystals and remineralization by calcium phosphate crystals in dentinal tubules. Those occurrences can expand the area at circumpulpal dentin and dentinal tubules with age[4-6]. On the other hand, a study showed no increasing trend found between the expansions of transparent dentin with age[7].

Analysis of transparent dentin is generally done by preparing the ground section of tooth and then observe under an optical microscope. The transparent area appears like glass under transmitted light or dark under reflected light. With the methods mentioned, measurement is made on a thin, hard-ground longitudinal section of the tooth and transilluminated with white light. On the other hand, analysis of transparent dentin was attempted by using radiation micro-CT without destroying the samples[8]. When polychromatic radiation having large energy spectra is generated, radiation passes through the sample and the beam hardening effect absorbs the radiation with low energy during x-ray transmission. When this happens, the x-ray becomes harder toward the center resulting to scattering rays. Together with other similar phenomena occurring at high energy radiation, it then becomes difficult to accurately measurement the mineral density due to the difference in density at the central and peripheral portions[9, 10].

Therefore, in order to create a non-destructive observation of transparent dentin and to avoid the artifacts caused by beam hardening, monochromatic radiation x-ray micro-CT (MR-μCT) in synchrotron radiation facility (SPring 8 by RIKEN, Hyogo) was used in this research. The accuracy of the method in observing hypercalcified dentin was compared to CMR (contact microradiography) with aluminum step wedge reference. Furthermore, the relationship between age and dentin hypercalcification was analyzed.

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Materials and Methods

This research was conducted with the approval of the Ethics Review Committee of Tsurumi University School of Dental Medicine (No. 1322).

Experimental Materials

A total of 44 human teeth extracted from 37 patients (22 males, 2 females and 2 unknown) ranging from 10th to 80th decade of life (19 to 88 years old) were used. These samples were donated to the Department of Oral Anatomy, Tsurumi University. After tooth extraction, the specimens were fixed in 10% neutral formalin solution,

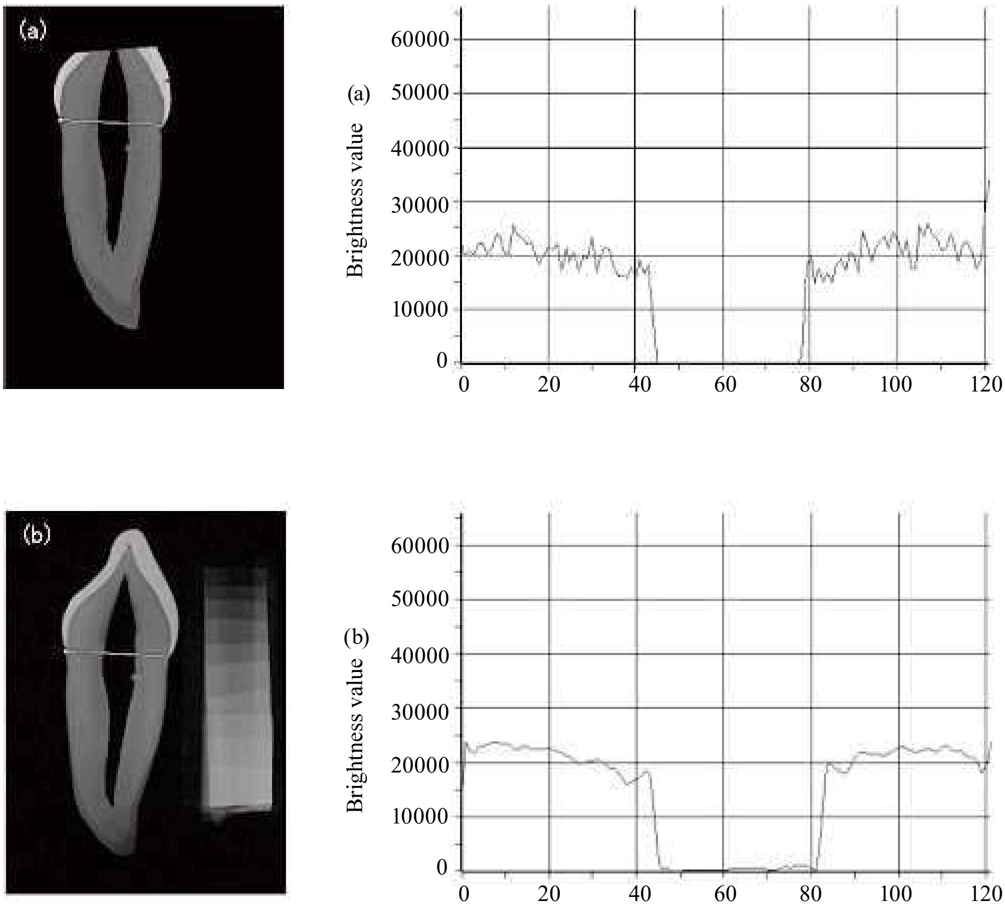

Figure. 1. Brightness value line analysis of MR-µCT and CMR images
a: Monochromatic optical radiation micro-CT, b: CMR image Brightness value line analysis (brightness value at 60,000 gradations) at cement-enamel junction with monochromatic radiation X-ray micro-CT (MR-µCT) and CMR images

then washed and rinsed with tap water overnight.

Experimental methods

Measurement of mineral density concordance ratio in MR-µCT and CMR

MR-µCT imaging

Forty-four human teeth consisting of 21central incisors, 14 lateral incisors, 4 canines, 2 premolars and 3 molars were photographed using MR-µCT in a synchrotron radiation facility (Beam Line; BL20B2), Super Photon ring-8 GeV (SPring8) located in Harima Science Park, Hyogo Prefecture. The photograph conditions were: 22 keV, 100 µA, at 60 min for samples with a root width of less than 5 mm and 30 keV, 100 µA and at 60 min for samples with a root width of more than 5 mm.

Preparation of Ground Section

Six teeth were randomly selected from the specimens and were photographed using MR-µCT. Ground sections were prepared for about 100 µm in thickness, then photographed under transmitted light microscope.

CMR (contact microradiography) imaging

CMR imaging was carried out using soft x-ray generator (SOFTEX C Series, Softex) with the following tube conditions: tube voltage of 10 kv, tube current of 2.5 mA at 35 min with 10 aluminum step (12 µm/step) wedge reference.

Analysis of MR-µCT and CMR images

Based on the shape of the pulp chamber, the slice images on CMR and MR-µCT using a software (TRI/3D-BON, RATOC) were superimposed. The MR-µCT resolution and brightness value (60000 gradations) were corrected to create a comparative image (Fig.1). A line analysis was performed to obtain the mineral density on MR-µCT and CMR images along the cemento-enamel junction using image analysis/measurement software (Image-pro PLUS, Media Cybernetics) (Fig.1). The brightness graph obtained from each image was used for the concordance ratio, and the correlation rate (%) between MR-µCT and CMR was calculated.

The relationship between age and hypercalcified dentin

Quantifying the brightness value

Using the image software from MR-µCT data, an image was created longitudinally and labio-lingually at the middle portion of the tooth. The brightness of each pixel of the image was converted into gray scale at 256 gradations.

Extraction of hypercalcified dentin

Extracted teeth used for preparing ground sections were also photographed using polychromatic radiation x-ray micro-CT (PR-µCT) (InspeXio SMX-225CT, SHIMADZU) with a tube voltage of 140 kV and a tube current of 70 mA. For the CT image analysis, a calibration curve was prepared from a reference concentration mineral

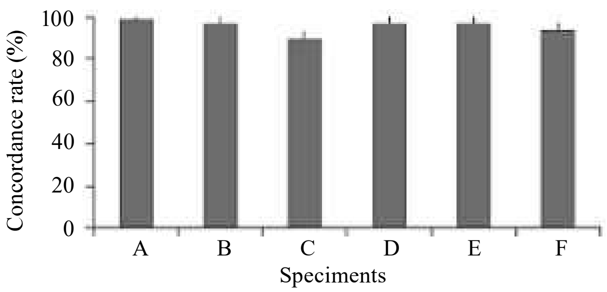

Figure. 2. The concordance rate of the mineral density corresponding to the MR-μCT and the CMR. The rate exceeded 90% in all 6 teeth. A ~ F expresses each 6 specimens.

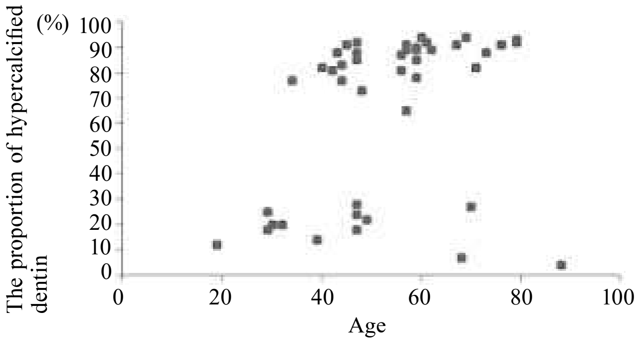

Figure. 3. Scatter plot of the percentage of hypercalcified dentin by age

standard phantom taken at the time of taking the photographs using measurement software (TRY/3D-BON, RATOC System Engineering co. LTD, Tokyo). Then after, the density of hypercalcified dentin area coinciding with the transparent dentin of the ground section was measured.

After converting the brightness of each pixel in CMR image with the brightness value of the aluminum step as an index, the Hydroxyapatite (HAp) density of the hypercalcified dentin was calculated using the following conditions: 1.54 angstroms of Copper Specific x-ray wavelength (Cu-Kα), a mass absorption coefficient of Hydroxyapatite at 83.5 and Aluminum at 49.0.

Therefore, the mass per unit of hydroxyapatite (g/cm^3) is obtained using the following formula:

mHA =(49.0/83.5)×mAl

mAl; the mass per unit of area of Aluminum of a sheet

And

mAl; =fAl×2.702×0.0012 (g/cm^2)

fAl; sheet number of Aluminum foil

Specific density of Aluminum; 2.702

Thickness of Aluminum foil; 12μm

Hence,

V(volume %) = (mHA/t)/3.15

Specific density of Aluminum; 3.15

t; thickness of the ground section (100μm=0.01cm in this case)

Therefore,

volume % of hydroxyapatite (HAp g/cm^3) = (49.0× (fAl × 2.702×0.0012)) / 83.5× 0.013.15

The volume % of hydroxyapatite of transparent dentin compared the brightness of an image equivalent to the transparent area using an optical microscope and was demanded from a brightness level of aluminum equivalent to the brightness of CMR image.

The density of the hypercalcified dentin area (HAp g/cm^3) was calculated from the PR-μCT and the CMR brightness using formulas mentioned above. In this study, a density above 1320~1350 HAp g/cm^3 was considered as hypercalcified dentin.

The CMR images were scanned with an optical digital scanner, the set of digital data of CMR with MR-μCT were collated and transparent dentin was identified with a brightness level. Including the hypercalcified dentin area, all dentin mineral density was converted into digital image brightness value (255 gradations). The image resolution compared each digital data with 20,000 pixels. After converting the image pixel data sets of MR-μCT image on all 44 teeth to be examined for the brightness value, the hypercalcified area in the root dentin was determined.

Comparison by age

The samples were divided into 3 groups based on generations: 10-30 years old, 40-69 years old and over 70 years. The number of specimens in each group is: 10-30 years old (n=8), 40-60 years old (n=30), 70 years and older (n=6). Then after, the area of hypercalcified dentin was compared between each group. In order to determine the difference between each type of tooth (i.e. incisor, canine, premolar, and molar), a comparison was carried out between central and lateral incisors in the 40-60's group.

The relationship between elapsed time (years) after root completion and hypercalcified dentin

Calculation of elapsed time after root completion.

The number of years from the age at tooth extraction to root completion in each type of tooth was taken as the elapsed time (years) after completion of the root.

Comparison by elapsed time

The specimens were divided into 3 groups based on elapsed time after root completion: 1-30 years (n=7), 31-60 years (n=30), over 61 years (n=7).

Calculation of the rate of increase in hypercalcified dentin per year

The average value of the percentage of hypercalcified dentin in each group was obtained by dividing the elapsed time after root completion by the average value of elapsed time to obtain the annual increase rate in each group.

Statistical analysis

The area of hypercalcified dentin between each group was compared and statistically examined by the difference of the mean values. Wilcoxon's Rank Sum Test was used for the elapsed time after root completion

Results

Measurement of mineral density concordance rate in MR-μCT and CMR images

Compared MR-μCT and CMR images of each set were considerably approximate with the mineral density obtained from the brightness value line analysis (Fig. 1). The concordance rate of the mineral density corresponding to the MR-μCT and the CMR exceeded 90% in all 6 teeth and the average was 95.6 ± 3.2% (Fig.2).

The relationship between age and hypercalcified dentin

MR-μCT image showed that the area of hypercalcified dentin in the root was age-specific (Fig. 3).

The area of hypercalcified dentin is 26.6 ± 22.7% in 10-30's;

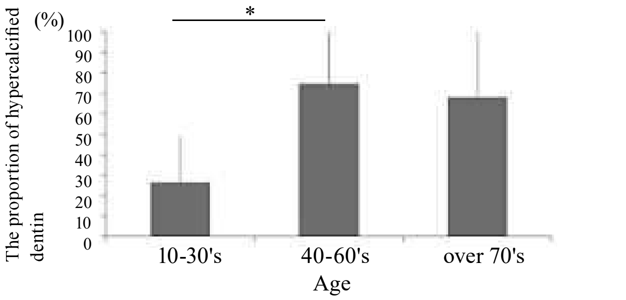

Figure. 4. Comparison of the percentage of hypercalcified dentin by age
A significant difference was observed between 10 to 30 and 40 to 60 years (*: $p<0.05$)

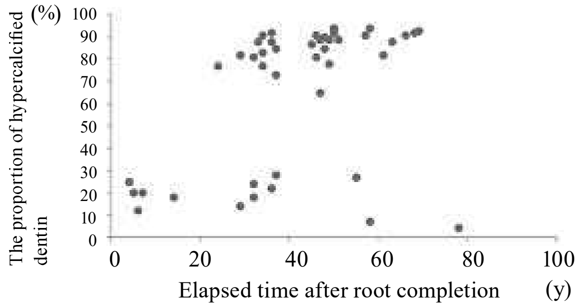

Figure. 5. Scatter plot of the percentage of hypercalcified dentin with elapsed time after root completion

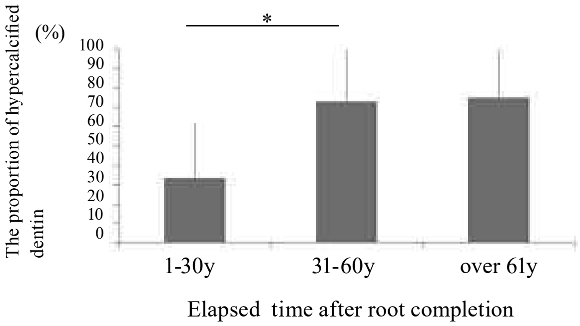

Figure. 6 Comparison of the percentage of hypercalcified dentin with elapsed time after root Completion
Significant difference was observed between 1 to 30 and 31 years or more (*: $p<0.05$).

74.8 ± 26.0% in 40-60's and 68.1 ± 36.8% in 70's and above. When comparing between two groups, the percentage of hypercalcified dentin was significantly higher in the 40-60's compared to 10-30's and no significant difference was obtained between 40-60's and over 70's ($p<0.05$) (Fig.4).

The area of hypercalcified dentin was compared according to the type of tooth. Moreover, the area of hypercalcified dentin in central and lateral incisors was compared using 14 centrals and 13 laterals in the 40-60's group. Results showed no significant difference between the central and lateral incisors in age groups ($p<0.05$). Neither a significant difference in the percentage of hypercalcified dentin was obtained ($p<0.05$).

Relationship between elapsed time after root completion and hypercalcified dentin

We measured the area of hypercalcified dentin area over a period of time from root completion to extraction (Fig. 5). The area of hypercalcified dentin was 33.4 ± 28.5% from 1-30 years, 72.9 ± 27.4% from 31-60 years and 74.9 ± 35.1% in over 61 years. When comparing each group, the percentage of hypercalcified dentin was significantly higher in 31-60 years and over 61 years compared to 1-30 years. However, there was no significant difference in between 31-60 years and over 61 years ($p<0.05$) (Fig. 6).

Survey on the rate of increase in the ratio of hypercalcified dentin per year

The rate of increase in hypercalcified dentin over one year were 2.3% for all data from 1 to 30 years, 1.7% in 60 years in 31-60 and 1.1% in 80 years for over 61 years.

Discussion

The issue with conventional method in studying transparent dentin using optical microscope is the destruction of sample during preparation of ground section. On the other hand with polychromatic x-ray micro-CT (PR-μCT), accuracy of hard tissue measurement is compromised due to the influence of scattered rays and beam hardening. Hence, measuring the mineral density is difficult with both methods. Considering these problems, hypercalcified dentin was measured using monochromatic x-ray micro-CT (MR-μCT). Extracted teeth were used to investigate the age, tooth type, years after root completion and distribution of hypercalcified dentin. In this study, the mineral density on MR-μCT and traditional CMR images were compared. The concordance rate is around 95% and over 90% in all samples indicating a high concordance rate. From this result, it was confirmed that measurement of hypercalcified dentin using MR-μCT is an effective method of measuring hard tissue without destroying the specimen. However, several factors may have influenced for the failure to reach 100%. Among the many factors were beam hardening effect caused by radiation source, geometric error of work table, motion error of work table, detection system, physical properties of samples, measurement condition, environmental and data processing, so on[11]. It was considered, however, that evaluation similar to that of was possible with PR-μCT performing correction for each subject, because with respect to these factors, the current PR-μCT has been properly corrected.

In this study, the deflection was accepted every generation on age distribution of the samples. There were 7 samples from 10-30 years old, 30 samples from 40-60 years old and 7 samples from over 70 years. Most samples were comprised of 40-60 years old. This can be explained by the belief that ages between 40-60 years is the highest in terms of Japanese population composition[12] and mortality significantly increases after those age[13]. In this regard, other factors should also be considered including the association with periodontal disease which is a major cause of tooth extraction.

Various structures are observed in dentin using optical microscope [14-26]. These structures are seen as transparent or opaque with visible light. Typically, these optical structures have been associated with mineral deposits although their definition is ambiguous[27]. Therefore as a definition, it was not distinguish transparent or opaque structures by optical lights methodologically, it was based on the calcium phosphate crystal deposition amount to dentinal tubules, intra- and inter-tubular dentin[22, 25, 26]. Then, the density above 1320-1350 HAp g/ cm^3 was used as hypercalcified dentin.

There are some reports on the relationship between circumpulpal dentin and aging[28] but the correlation is unknown. Dentin sialoprotein

Table 1. The age of each prediction section and the ratio of hypercalcified dentin
The area of hypercalcified dentin to the root dentin was determined for each prediction interval (mean ± standard deviation)

	±1SD	±1.5SD	±2SD
10-30's	18.1±4.7%	18.1±4.7%	18.1±4.7%
40-60's	85.8±7.1%	85.8±7.1%	79.2±20.4%
over 70's	89.1±4.3%	78.8±25.7%	68.1±36.7%

(DSP)[29] is localized in circumpulpal dentin, and Yamakoshi *et al.* reported that DSP contains a large amount of chondroitin sulfate[30, 31]. These reports suggested that circumpulpal dentin controls moisture through the presence of chondroitin sulfate. Moreover as DSP decreases with aging, the moisture control would be lost, and possibly that changes in circumpulpal dentin may have been affected by aging.

In this study, we examined the correlation between calcification of dentin and age using MR-μCT. Gustafson proposed the correlation between dentin calcification and age in addition to occlusion, secondary dentin formation, cementum and transparent root dentin as an index of age estimation[1-7, 32, 33]. However, when we classify the related literature, there are three categories: 1) there is a certain correlation between transparent dentin and age; 2) that almost no correlation was observed; 3) that the correlation is unknown. Accordingly, we considered that it is necessary to further examine the relationship between hypercalcified dentin and age by application of x-ray source instead of analyzing transparent dentin and age.

The results of this study showed an increasing tendency of hypercalcified dentin in x-ray with high significant difference between 10-30 years and 40-60 years. Nevertheless, no significant difference was obtained between 40-60 years and over 70 years ($p<0.05$).

On the other hand, Bang, G et al measured the amount of transparent dentin formed along the length in 1013 human extracted teeth and measured the amount of transparent dentin formed at the root. They found a strong correlation between the amount of transparent dentin and age in all types of teeth[32]. The study showed a rapid increase after 40-50 years. Hence, it was considered that physiological metabolism in humans is involved in the formation of hypercalcified dentin including transparent dentin, and the amount of calcification slows down as metabolism decreases in over 70 years old. The relationship between the rate of increase in hypercalcified dentin and age distribution in all samples used in this study was prudently examined and interesting findings were obtained.

The data of the three prediction intervals (±1 SD, ±1.5 SD, ±2 SD) obtained from the mean value and standard deviation are shown in Table 1. The ratio of hypercalcified dentin is calculated from the average value of each age group in all specimens. There were 8 specimens outside the ±1 SD range, of which 7 specimens appeared outside the ±1.5 SD range and 3 specimens were outside the ±2 SD range. Based on the results, the area of hypercalcified dentin shows an increasing trend with aging although there were few individuals who extensively deviated from the average. For instance, even though different types of teeth extracted from the same person were included, the same tendency was confirmed in the same person. Nevertheless, variations among individuals were also noted.

In this study, the area occupied by hypercalcified dentin in individuals over 70 years old was as high as 68.1% and it was even higher at 85.8% in the data within the ±1.5 SD range (87% predicted). From this, it was confirmed that in elderly people over 60 years old, the hypercalcified dentin area including the transparent dentin area occupied 70% or more of the root.

Eto *et al.* reported the differences in the rate of increase in transparent dentin between the types of teeth using 122 human anterior teeth aged 16 to 69. Among the reports on transparent dentin and age so far, differences between tooth types have also been studied. Specifically, at age 40-49 years old, increasing amount of transparent dentin in central incisor was significantly higher than in lateral incisors and cuspid. However, it was reported that after 50 years old, the central incisors start to slow down while the lateral incisors and cuspids begin linear increase after the age of 50[33]. In this study, there was no significant difference between the central and lateral incisors at 40-60 years old. However, for data within ±1 and ±1.5 SD range, the area of the hypercalcified dentin area in lateral incisors was significantly higher than in central incisors. Thus, it was confirmed that the area of hypercalcified dentin area varies depending on the type of tooth in about 87% of the population. Further studies increasing the number of samples are necessary.

In this study, the elapsed time after root completion was calculated by subtracting the tooth root age[34, 35] from the extraction age and the relationship with the area of hypercalcified in the root dentin was examined. This can be explained by increased mineralization in root dentin which happens at the root apex after receiving occlusal force. Similarly, it was confirmed that there are more transparent dentin on the apical side than on the coronal side. Saruwatari et al. sectioned 90 human maxillary anterior teeth from 11 to 76 years old at positions 1/4, 1/2, 3/4 from the root apex. They found out that the highest ratio of transparent dentin is at 1/4 from apical side[36]. This tendency was confirmed by the presence of abrasion on specimen, which was further supported by a higher value of brightness on the apical side. Thus both transparent dentin and hypercalcified dentin were formed from the apical side of the tooth. Azaz et al studied 72 human canines and mentioned that the area of transparent dentin increases with age even in impaired teeth[37]. From these facts, in order to see the correlation, it is necessary to include occlusal force to determine the ratio of hypercalcified dentin area in each type of tooth and to take into consideration the type of tissue present in the tooth.

When the yearly rate of increase in hypercalcified dentin was calculated, the rates obtained were: 1.7% for 31 to 60 years after root completion, 2.3% for 1 to 30 years after root completion and the lowest value obtained was 1.1% for over 61 years. According to the elapsed time, the rate of increase in hypercalcified dentin per year has a tendency to drop. This was thought to be due to the decrease in metabolic function associated with physiological changes due to aging.

In this study, as shown in Fig. 3 and Fig. 4, the ratio of hypercalcified dentin was greatly dissociated into two groups showing values of around 80% and 20%. This could be explained by the involvement of many factors such as origin of the sample and size of the population. However, since no specimen with a value of around 80% was between 10 to 20 years, it is suspected that the tooth extraction was carried out due to pathological reason. Yet, those influencing factors are the exact opposite of the conclusions in previous reports.

The present study conferred the age estimation in transparent dentin which can be applied in the field of dental science. The relationship between hypercalcified dentin and age were shown by the values obtained by utilizing monochromatic optical radiation x-ray micro-CT. Furthermore, the study enabled us to obtain a certain reference value for extraction and measurement of hypercalcified dentin in a non-destructive way by multi-wave optical radiation x-ray micro-CT. Moving forward, quantitative measurement of dentin calcification status by micro-CT will become more convenient and possible. Hence, this would contribute to the accuracy in estimating the individual age legally in the dental field. Further studies may be carried out by increasing the number of samples.

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Conflict of Interest

The authors declared no conflict of interest.

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