

RESEARCH ARTICLE

Enamel and dentin mineralization in familial hypophosphatemic rickets: a micro-CT study

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Objectives: The aim of the present study was to analyse the mineralization pattern of enamel and dentin in patients affected by X-linked hypophosphatemic rickets (XLHR) using micro-CT (μ CT), and to associate enamel and dentin mineralization in primary and permanent teeth with tooth position, gender and the presence/absence of this disease.

Methods: 19 teeth were collected from 5 individuals from the same family, 1 non-affected by XLHR and 4 affected by XLHR. Gender, age, tooth position (anterior/posterior) and tooth type (deciduous/permanent) were recorded for each patient. Following collection, teeth were placed in 0.1% thymol solution until μ CT scan. Projection images were reconstructed and analysed. A plot profile describing the greyscale distance relationship in μ CT images was achieved through a line bisecting each tooth in a region with the presence of enamel and dentin. The enamel and dentin mineralization densities were measured and compared. Univariate ANOVA and *post hoc* Tukey tests were used for all comparisons.

Results: Teeth of all affected patients presented dentin with a different mineralization pattern compared with the teeth of healthy patients with dentin defects observed next to the pulp chambers. Highly significant differences were found for gray values between anterior and posterior teeth ($p < 0.05$), affected and non-affected ($p < 0.05$), as well as when position and disease status were considered ($p < 0.05$).

Conclusions: In conclusion, the mineralization patterns of dentin differed when comparing teeth from patients with and without FHR, mainly next to pulp chambers where areas with porosity and consequently lower mineral density and dentin defects were found.

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Introduction

Familial hypophosphatemic rickets (FHR) is a rare condition with an incidence of 1 in 20,000 births.¹ Several forms of FHR have been described, distinguished by their pattern of inheritance and genetic cause. The most common form of FHR is known as X-linked hypophosphatemic rickets (XLHR), which has

an X-linked dominant pattern of inheritance.² Mutations in the phosphate-regulating gene with homologies to endopeptidases on the X-chromosome are responsible for the disease in most familial cases.³ The other forms of FHR are rare, and include X-linked recessive, autosomal dominant, autosomal recessive and hereditary hypophosphatemic rickets with hypercalciuria.²

Clinically, XLHR is characterized by short stature, deformities predominantly in the lower limbs, metaphyseal widening, rachitic rosary and frontal bossing.⁴ At birth,

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children present normal height, but growth rate reduction is present during the first year of life, resulting in a final deficit in height, which is a consequence of the slow speed of growth that precedes the diagnosis and initiation of therapy.⁵⁻⁷ The differential diagnosis from “vitamin D deficiency rickets” is based on the non-compliance with hypotonia, myopathy, muscle weakness or tetany.⁸

The recurrence of spontaneous abscesses affecting multiple non-carious primary and permanent teeth is the main oral clinical feature associated with FHR.^{9,10} Teeth are characterized by deficient dentin mineralization with large pulp chambers¹¹ and the presence of large pulp horns and structural defects in the dentin.¹² McWhorter and Seale¹³ suggested that abscess formation occurs secondary to bacterial invasion into the pulp through microcracks present in the enamel and dentin. Histological sections of the affected teeth show dentin disorganization.¹⁴ Radiographically, dental characteristics are generally evaluated through periapical¹⁴ or panoramic radiographs.^{9,12,15} Scanning electron microscopy and immunohistochemistry have also been used to assess the primary and permanent dentitions of patients with FHR.^{16,17}

X-ray micro-CT (μ CT) is a high-resolution, non-destructive radiographic method, which has been increasingly used in studies of dental anatomy.^{18,19} Described by Elliot and Dover,²⁰ μ CT is a microscopic system based on the principles of CT, which interprets X-ray attenuation variation within a planar section of a solid object, without the need to make cuts. This technique allows microstructural analysis of small bodies, enabling the study of parameters, such as mineral density,¹⁸ volume²¹ and mineralization pattern.^{19,22} Although μ CT is an important technology in microstructure studies, no previous work has reported its use in teeth of patients diagnosed with XLHR. The aim of the present study was to analyse the mineralization

pattern of enamel and dentin in a family of patients affected by XLHR using μ CT, and to verify variations in enamel and dentin mineralization in primary and permanent teeth, as a function of tooth position, gender and the presence/absence of this disease.

Methods and materials

Samples

Five individuals from the same family (one non-affected and four affected by XLHR) donated 19 teeth to be used in the present study. These teeth were previously extracted for reasons other than the present study. Hence, the primary cause for extractions was the presence of either periodontal disease or dental caries. The following characteristics were recorded for each patient: gender, age, tooth position (anterior—incisors, canines; posterior—premolars, molars) and tooth type (deciduous/permanent) (Table 1). Following collection, teeth were placed in 0.1% thymol solution until μ CT scan. This study was approved by the Ethics Committee of the Federal University of Ceará Medical School (Brazil) (Protocol 004/11). Consent and tooth donation forms were signed by volunteers or legal guardians, prior to patient enrolment in the study.

micro-CT analysis

High-resolution μ CT (SkyScan 1172; SkyScan, Antwerp, Belgium) was used to scan all teeth. Samples were wrapped in Parafilm[®] (Bemis NA, Neenah, WI) as a way to prevent drying and affixed to the scanning stage by the use of this same plastic paraffin film. Projection images were obtained using the following scanner settings: voltage, 70 kV; resolution, 8.9 μ m; aluminium filter, 0.5 mm; stage rotation, 0.4° per second; and frame averaging, two. Flat-field corrections were

Table 1 Teeth distributed by gender, age and the presence/absence of X-linked hypophosphatemic rickets (XLHR) of study volunteers

Individuals	XLHR	Gender	Age (years)	Tooth	Tooth position	Tooth type	Greyscale value (mean \pm standard deviation)	
							Enamel	Dentin
1	Absent	Female	13	1	Posterior	Deciduous	151.26 \pm 5.01	77.91 \pm 0.31
				2	Anterior	Deciduous	152.22 \pm 20.82	93.59 \pm 1.61
				3	Posterior	Deciduous	151.64 \pm 3.83	79.99 \pm 1.79
				4	Anterior	Deciduous	176.34 \pm 4.79	94.95 \pm 1.59
				5	Posterior	Deciduous	151.64 \pm 12.51	84.48 \pm 0.87
				6	Posterior	Deciduous	165.27 \pm 9.82	84.65 \pm 0.55
2	Present	Male	6	1	Anterior	Deciduous	158.83 \pm 30.44	73.06 \pm 5.95
				2	Posterior	Deciduous	148.30 \pm 21.41	61.64 \pm 7.02
				3	Anterior	Deciduous	168.64 \pm 11.77	69.09 \pm 4.86
				4	Anterior	Deciduous	173.17 \pm 8.08	76.40 \pm 3.88
				5	Anterior	Deciduous	161.50 \pm 15.82	78.40 \pm 3.47
				6	Posterior	Deciduous	167.88 \pm 3.89	83.63 \pm 2.42
				7	Anterior	Deciduous	157.16 \pm 23.47	66.56 \pm 2.79
				8	Anterior	Deciduous	178.60 \pm 11.89	76.54 \pm 7.47
				9	Anterior	Deciduous	161.19 \pm 9.25	86.77 \pm 2.24
3	Present	Female	10	1	Posterior	Deciduous	159.42 \pm 5.39	83.05 \pm 1.50
4	Present	Female	31	1	Posterior	Permanent	140.79 \pm 10.69	73.90 \pm 1.26
5	Present	Male	27	1	Posterior	Permanent	163.44 \pm 5.55	71.58 \pm 2.03
				2	Anterior	Permanent	149.72 \pm 22.52	72.64 \pm 2.82

used to minimize background noise. Projection images were reconstructed through manufacturer-provided software (NRecon; SkyScan) with the following settings: beam hardening, 33%; ring reduction, 5% and threshold, 0.00–0.13. In addition, post-alignment was optimized separately for each specimen.

Reconstructed images were analysed with the ImageJ 1.43u software (Wayne Rasband, National Institutes of Health, Bethesda, MD). A plot profile describing the greyscale distance relationship in μ CT images was achieved through a line bisecting each tooth at a standardized cross-sectional occlusal level with the presence of enamel and dentin. In each tooth, five lines were drawn and the average of greyscale values for enamel and dentin was calculated. The enamel and dentin mineralization densities were measured and compared.

Statistics

All statistical tests were performed with SPSS[®] 9.0 software (SPSS Inc., Chicago, IL). Univariate ANOVA was used for all comparisons, with either enamel or dentin greyscale as the dependent variable, and variables such as gender, age, tooth position, tooth type and the presence/absence of XLHR as the independent variables. *Post hoc* Tukey tests were only used when more than two groups were being compared. In these cases the *post hoc* Tukey test was applied. Values were considered significant when $p < 0.05$.

Results

The dentin mineralization pattern differed between teeth of the unaffected and affected individuals (Figure 1). Dentin defects were observed next to the pulp chambers as areas with porosities and consequently lower mineral density. No visual differences were detected in pulp space and enamel. Based on a cross-section of each tooth, a bisecting line was established from enamel towards the pulp. Mineralization patterns were expressed as greyscale–distance plot profiles along this line and are represented by histograms as shown in Figure 2. In Figure 2, higher peaks represent higher greyscale values, so that enamel shows the highest values, followed by dentin and pulp. In addition, parallel to the x -axis in each histogram, a more stable line can be observed in the dentin of the non-affected patient. This finding was not observed among affected individuals.

When considering tooth position and the presence/absence of XLHR, comparisons of enamel greyscale values (Table 1) differed only between anterior and posterior teeth ($p = 0.027$), but no difference was found between the affected and non-affected individuals ($p = 0.965$), nor when all these variables were considered ($p = 0.823$). However, when these comparisons were carried out with dentin greyscale value as the dependent variable, highly significant differences were found for grey values

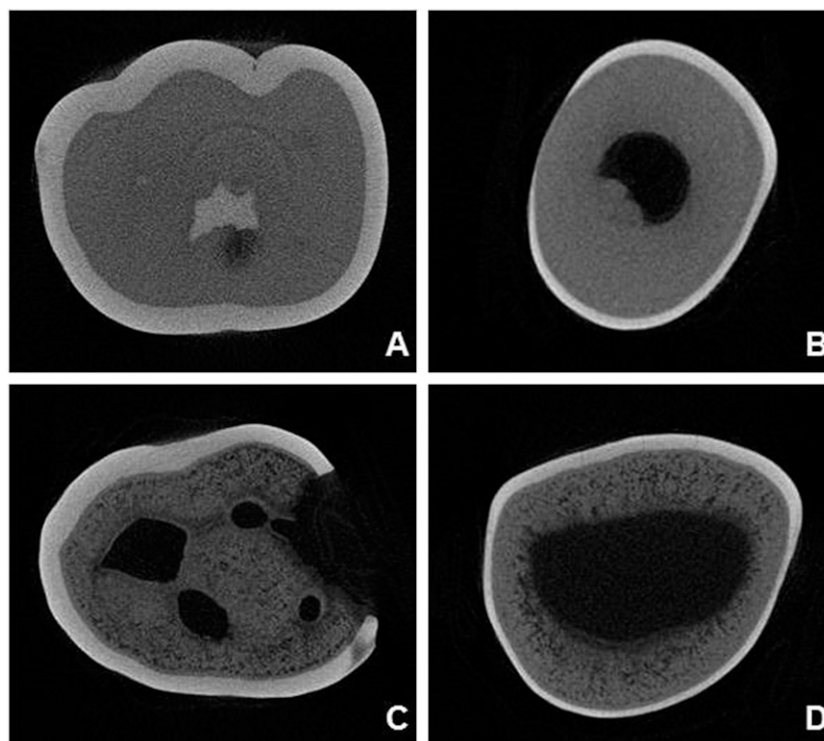


Figure 1 Two-dimensional micro-CT reconstructed images (NRecon; SkyScan, Antwerp, Belgium). Cross-section at a more occlusal level showing normal pattern of mineralization in non-affected individuals [(a) molar, (b) incisor]. Cross-section at a more occlusal level showing hypomineralization defects to be more widespread in dentin of affected individual [(c) molar, (d) incisor].

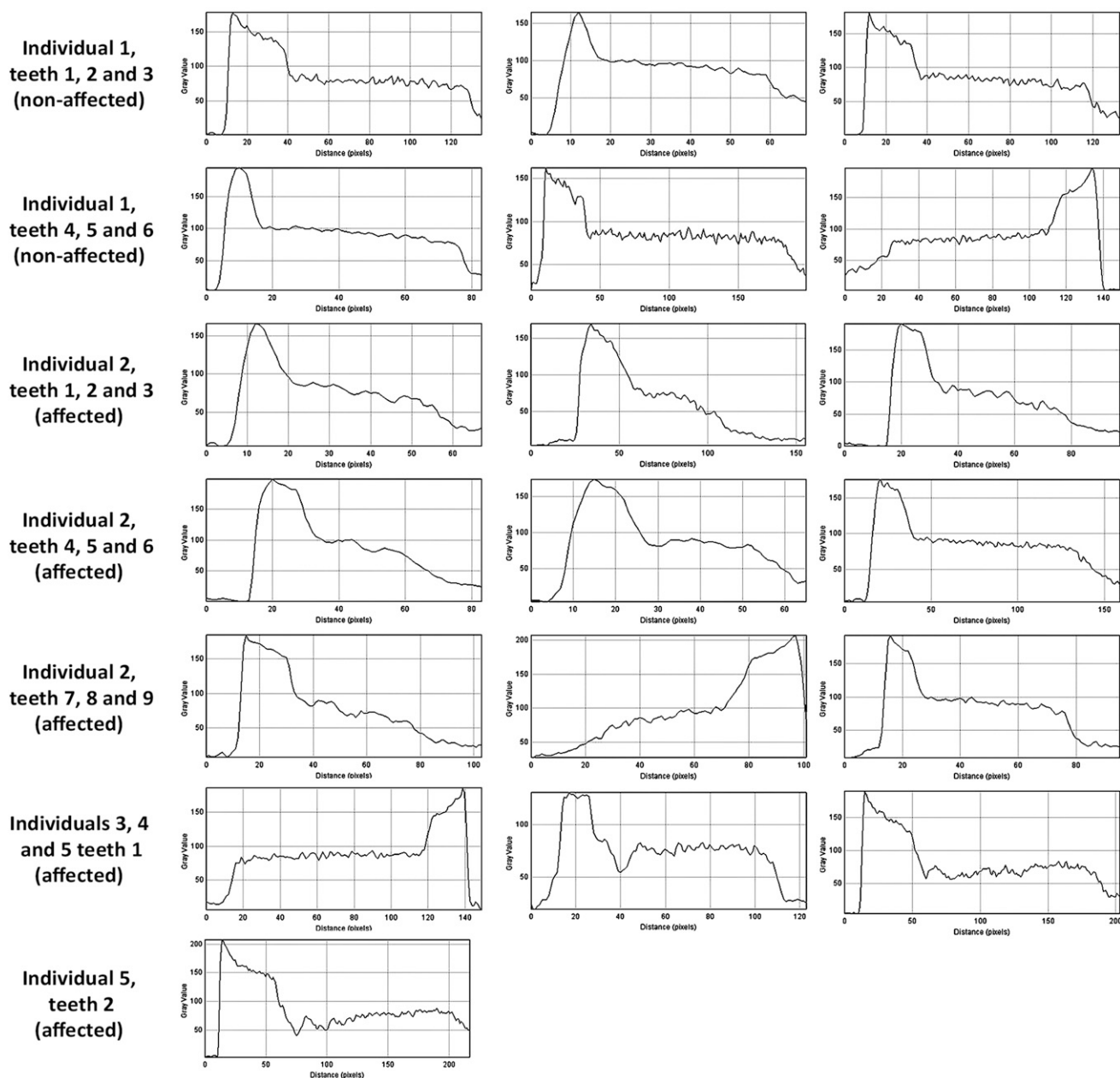


Figure 2 Histograms expressing mineralization patterns as greyscale–distance plot profiles along the line bisecting teeth of affected and non-affected individuals. Each histogram expresses mineralization patterns from enamel to pulp for: individual 1 (teeth 1–5), individual 2 (teeth 1–7 and tooth 9), individual 4 (tooth 1) and individual 5 (teeth 1–2). Mineralization patterns are expressed from pulp to enamel for: individual 1 (tooth 6), individual 2 (tooth 8) and individual 3 (tooth 1).

between anterior and posterior teeth ($p < 0.05$), affected and non-affected ($p < 0.05$), as well as when position and disease status were considered ($p < 0.05$).

Evaluation of dentin greyscale values (Table 1) and gender alone showed a significant difference between males and females ($p < 0.05$), whereas the female gender expressed the highest values of greyscale. However, enamel greyscale values did not differ between genders ($p = 0.057$). Dentin grey values differed between dentitions ($p < 0.05$), hence deciduous teeth presented higher greyscale values than did permanent teeth.

Discussion

μ CT is a miniaturized version of medical CT and has been used extensively for *in vitro* dental research.^{23–26} The technique allows three-dimensional analyses of both structure and density (or concentration), the latter requiring prior knowledge of composition.²⁷ Although μ CT was not previously used to assess the mineral structure of teeth from patients with rickets, other studies have focused on the tooth mineral structure from these individuals by using scanning electron microscopy,^{17,28}

transmission electron microscopy,²⁹ immunohistochemical examination^{16,17} and radiographic examination.¹⁴

Teeth of all affected patients presented dentin with a different mineralization pattern compared with teeth of the unaffected individual. Dentin defects were noted next to pulp chambers as areas with porosities and consequently lower mineral density. This fact was confirmed by dentin greyscale values, which differed between affected and non-affected patients. Thus, a higher number of porosities renders a lower mineral density, numerically observed as reduced greyscale values. Porosities observed in the present study reflect the presence of dentinal canals, which are usually uniform and highly aligned, expressing a regular distribution in healthy teeth. Since dentin is a very anisotropic tissue, it tends to deform differently when loaded along *vs* across tubule orientation.³⁰ In the present study, teeth from affected individuals expressed lower greyscale values in dentin when compared with the non-affected patient. This finding may result from a greater number and a more irregular distribution of these dentinal tubules in the teeth of affected subjects. Although odontoblast function is normal in patients with XLHR, dental mineralization is inadequate. Hence, hypophosphatemia, which occurs as a consequence of XLHR, generates a dysplastic and poorly mineralized dentinal tissue with areas of interglobular dentin.¹² Interglobular dentin and irregular dentinal tubules were histologically observed by Pereira *et al*.¹⁴

In the present study, no visual differences were detected in enamel mineralization and pulp chamber. Enamel greyscale did not differ between affected and non-affected individuals. Harris and Sullivan,³¹ and Archard and Witkop³² also described the enamel of individuals with FHR as normal but thin. However, enamel hypoplasia has been reported in several studies.^{12,33,34} Large canals and root canal space were also previously described.¹⁴ These abnormalities may explain the common outbreak of periradicular abscesses in the absence of caries or history of trauma, typically found in FHR, caused by bacterial contamination *via* enamel microfractures, which extend to the pulp and often lead to tissue necrosis.

Enamel and dentin greyscales differed between anterior and posterior teeth in the presence and absence of XLHR. Sánchez-Quevedo *et al*³⁵ evaluated human teeth with amelogenesis imperfecta through scanning electron microscopy and X-ray microprobe analysis. The authors analysed dental fragments from members of a family clinically and genetically diagnosed as having amelogenesis imperfecta to establish the morphological patterns and the quantitative concentration of calcium in the enamel of anterior and posterior

teeth. Calcium levels in the enamel of teeth with and without amelogenesis imperfecta differed significantly between anterior and posterior teeth, indicating that the factors that influence normal mineralization in different regions of the dental arch are not altered in the process of amelogenesis imperfecta.³⁵ However, in the present study, the mineralization level of dental tissues was assessed by quantifying greyscale in both enamel and dentin, and the results showed significantly higher mineralization levels in the analysed posterior dentition.

In this study, female patients affected by XLHR presented a higher mineral density in dentin than did males. Winters *et al*³⁶ studied hypophosphatemia in a large North Carolina family of English–Scottish descent. They observed that the degree of serum phosphate reduction was the same in males and females, although females expressed less severe bone disease. Dentin defects reflect genetic disorders affecting bone mineralization, such as hypophosphatemic rickets,³⁷ and our results agreed with Winters *et al*.³⁶ In a healthy population, although males tend to present a 10–12% peak bone mass and greater bone size, density is similar in males and females.³⁸

Deciduous teeth expressed the most elevated greyscale values, suggesting a higher mineralization pattern in the dentin of primary than in permanent dentition. This finding is consistent with the study of Hirayama *et al*³⁹ that reported that peritubular dentin thickness, which is the dentin with higher mineral content, was two to five times greater in deciduous teeth, with values ranging from 3 to 20 μm , than in permanent teeth. Also supporting our finding, Sumikawa *et al*⁴⁰ reported that tubule diameters increased with distance from the dentin–enamel junction, whereas a decrease in peritubular width was observed.

Enamel greyscale values did not differ between deciduous and permanent dentitions. Wilson and Beynon⁴¹ measured, by quantitative microradiography, mineralization and compared differences in mineralization between human deciduous and permanent enamel. The authors found an overall lower mineralization pattern in the deciduous dentition in addition to lower mineral levels in the deciduous enamel.⁴¹

In the present sample, patients with XLHR showed lower dentinal and enamel mineralization than did unaffected individuals from the same family. Mineralization pattern varied among these patients as a function of gender, tooth position and type of dentition (deciduous or permanent). The utilization of μCT provided a detailed visualization of enamel and dentinal microdefects, commonly observed among patients with XLHR.

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