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Case Report and X-ray Microtomography and Scanning Electron Microscopy Analysis of Teeth with Hypocalcified Amelogenesis Imperfecta

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CASE REPORT

Case Report and X-ray Microtomography and Scanning Electron Microscopy Analysis of Teeth with Hypocalcified Amelogenesis Imperfecta

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ABSTRACT

Amelogenesis imperfecta (AI) is a genetic disease characterized by producing morphological and structural changes in tooth enamel. **Objective:** To highlight the morphological changes observed in teeth with hypocalcified AI based on scanning electron microscopy and microtomography analysis. **Case report:** An adult male had teeth seriously compromised by hypocalcified AI. The treatment plan involved removal of third molars followed by restorative and prosthetic treatment. After extraction, the teeth were analyzed by SEM and X-ray microtomography (microCT). Teeth with AI revealed the presence of microfractures arranged from the outer surface to the center of the enamel. Microfractures had a cracked or "cracked ground" pattern. SEM confirmed the presence of microfractures and distortion in the orientation of the enamel prisms in some regions. **Conclusion:** Teeth of patients with hypocalcified amelogenesis imperfecta IIIA subtype show significant structural changes that are closely related with the ease of fractures.

Key words: amelogenesis, amelogenesis imperfecta, dental enamel, electron, microscopy, scanning, x-ray microtomography

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INTRODUCTION

Amelogenesis imperfecta (AI) is a term used to define a group of conditions with heterogeneous clinical and genetic features that affect dental enamel. In some cases, patients may manifest other changes in teeth, oral and extraoral tissues.[1] The prevalence of this condition is low and has varied in different populations (0.00025 – 0.43%).[2-5]

AI can have four different clinical manifestations that can be divided into 15 subtypes. Each subtype exhibits a specific clinical manifestation ranging from inadequate thickness to complete absence of enamel.[6] Several classification systems for AI have been proposed based on the alterations suffered by the teeth. However, the most accepted is the Witkop classification. According to this classification, AI can be classified as hypoplastic, hypomatured, hypocalcified and hypomaturated-hypoplastic.[7] Patients with hypoplastic amelogenesis imperfecta exhibit multiple small defects spread across the tooth surface. In the hypomaturated type, the affected teeth present with mottling, opaque white-brownish yellow discoloration, and is highly brittle. The hypocalcified type manifests pigmented, softened, and loosened enamel. Finally, teeth with areas of enamel hypoplasia in combination with hypomaturation characterize the type hypomaturated-hypoplastic.[6]

The enamel is properly deposited during its formation, but adequate mineralization is not present in amelogenesis imperfecta of the hypocalcified type. Therefore, when the teeth erupt in the mouth, they present only with an altered color. They usually exhibit a coloration that varies from yellow to light brown, but quickly assume a darker coloration. Enamel is easily fractured during masticatory function due to incorrect mineralization. In this way, over the years, tooth enamel ends up being lost in whole or in part. An interesting fact is that the enamel in the cervical portion is occasionally better calcified.[6]

The treatment of AI depends on each case, but it is important to consider the functional and psychological impact of the patient.[9,10] The literature describes several case reports with different forms of treatment.[9-16] In general, a multidisciplinary approach is necessary in therapeutic planning.[11-13]

High-resolution X-ray microtomography (microCT) allows the analysis of biological structures from a three-dimensional at micrometer to submicrometer resolution.[17] Scanning electron microscopy (SEM) remains distinct in its ability to allow topographical visualization of structures.[18] The purpose of this article is to report a case of hypocalcified amelogenesis imperfecta (autosomal dominant subtype IIIA) and the morphological characteristics of the teeth obtained by microCT and SEM.

CASE REPORT

A 22-year-old white man came to the University's dental clinic complaining of poor appearance of his teeth. According to the patient, the teeth are ugly to the point of compromising his socialization. During the anamnesis, he reported that two other brothers had the same problem with their teeth. The patient reported that the teeth erupted normally into the mouth but broke easily. The patient did not have any systemic disease and did not use medication.

No changes in the oral and extraoral soft tissues were observed on physical examination. On the other hand, examination of the teeth revealed that the patient had composite resin restorations on the buccal side of the maxillary incisors, canines and premolars (Figure 1). The upper posterior teeth exhibited a serious impairment of esthetics and function due to total loss of enamel with exposure of dentin (Figure 1).

Enamel was still intact on the cervical third of the buccal surface of the posterior teeth even after total loss on the other surfaces. The teeth had a color that varied from light brown to black. The standard dental anatomy was still preserved in some teeth. However, other teeth already had a severe loss of enamel and, consequently, of dentin. Despite all dental alterations, the patient did not report pain or discomfort.

A panoramic radiograph revealed an important loss of tooth structure, the presence of impacted teeth (Teeth: 18, 38 and 48), agenesis of upper left molar and taurodontism in right and left upper molars (Figure 2).

The patient's older brother was brought in for consultation later and also had a similar dental appearance. Thus, the diagnosis of AI was established based on clinical and radiographic information.

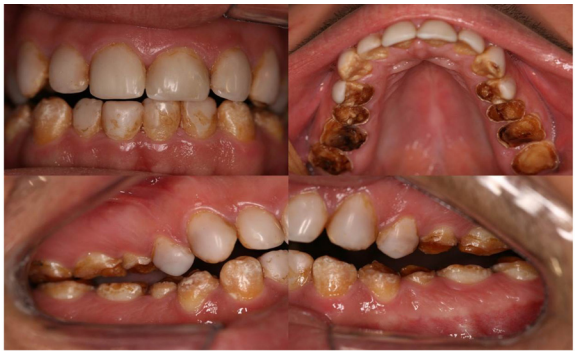

Figure 1. Teeth with hypocalcified amelogenesis imperfecta.

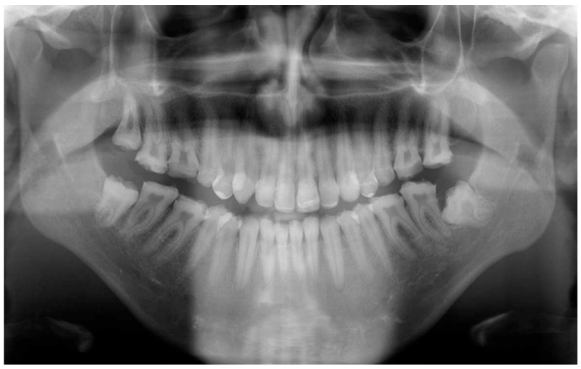

Figure 2. Radiographic image of teeth with hypocalcified amelogenesis imperfecta.

The established treatment plan initially provided for the surgical removal of teeth 18, 38 and 48 before restorative and prosthetic treatment. The three teeth were collected during the surgical procedure and sent for analysis at the Mineral and Rock Analysis Laboratory at the University. Third molars from patients without amelogenesis imperfecta were sent for analysis as controls. The patient signed an informed consent form so that his teeth could be analyzed in the laboratory.

MicroCT analysis

In the laboratory, the teeth were clean and dried using a 402/3N drying oven (Ethiktechnology, São Paulo) at 50°C for 12 hours (Figure 3). Then, each tooth was placed on a styrofoam base and analyzed in the Skyscan 1172 microtomograph (Bruker, Belgium). The image capture was performed using the source potential and current of 89 kV and 112 µA, respectively.

The pixel size used was 5.03 µm without the aid of filters with an exposure time of 1,200 ms per projection image. The images were generated by a CCD charge couple device camera and captured by an image detector with a resolution of 1,336 x 2,000 pixels. The sample rotation was 0.27° per image and the total sample rotation was 180°. The total acquisition time was 1 hour.

Figure 3. Teeth with hypocalcified amelogenesis imperfecta - view after surgical removal.

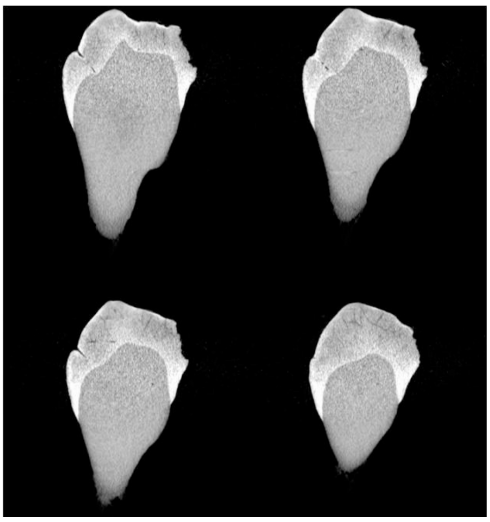

Figure 4. MicroCT sections showing the direction of the microfractures.

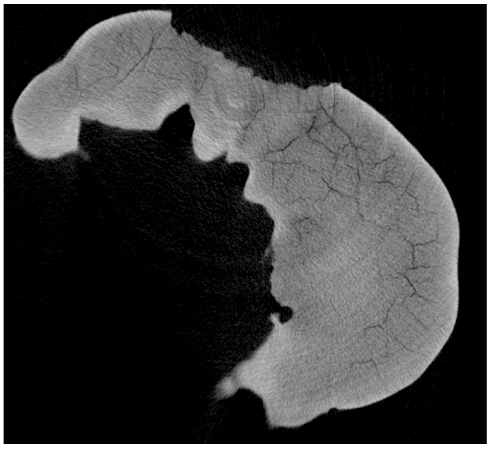

Figure 5. MicroCT section showing the pattern of microfractures.

The tomographic sections of the three teeth with amelogenesis imperfecta revealed the presence of microfractures arranged in the direction from the outer surface of the enamel towards the center of the tooth (Figure 4). The arrangement of microfractures assumed a cracked or "cracked ground" pattern (Figure 5). On the other hand, no microfractures were found in the enamel of the cervical region. Microfractures were more evident in the upper and middle thirds of the crown (Figure 5).

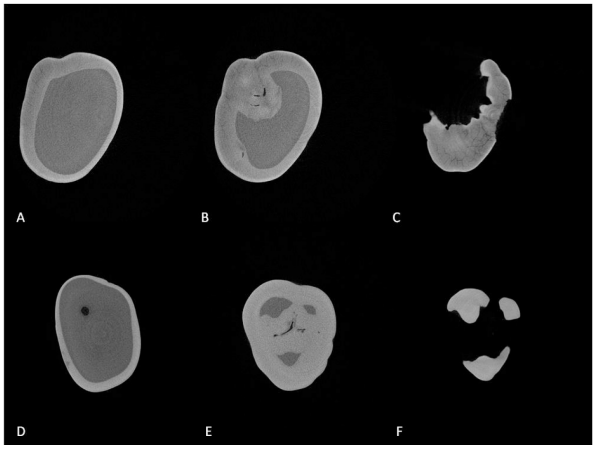

Figure 6. A, B and C. MicroCT sections showing the arrangement of microfractures in the middle and occlusal thirds of defective teeth amelogenesis. D, E, and F. MicroCT sections of control tooth (No microfractures).

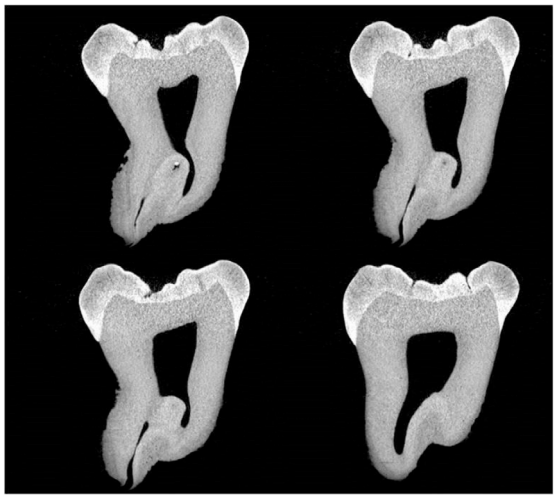

Figure 7. Teeth with amelogenesis imperfecta and taurodontia viewed by microCT.

In addition, some areas of defects in the form of dots in the enamel were observed on the occlusal, buccal and mesial surfaces (Figure 6). MicroCT analysis showed that enamel thickness was preserved both in teeth affected by AI and in controls (Figures 6A and 6D). MicroCT analysis of the control teeth did not show any significant alteration, or images of microfractures or superficial defects (Figures 6C to 6F). Taurodontia was confirmed by microtomography as shown in figure 7.

SEM analysis

Each tooth was sectioned in half using a Discoplan-TS precision cutting machine (Stuers, Denmark). One half of the crown of the teeth was kept intact for analysis by SEM. This morphological analysis was performed with the aid of the electronic microscope JSM 6010LA (JEOL, Japan). The other half of the

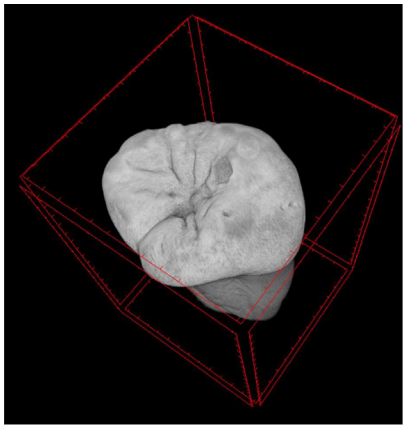

Figure 8. Three-dimensional reconstruction by microCT showing punctate defects of enamel.

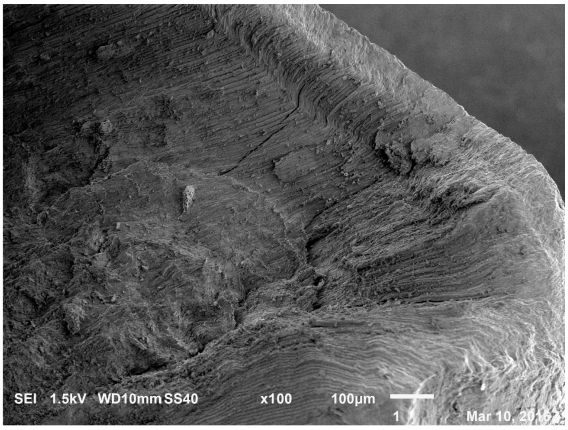

Figure 9. Scanning electron micrograph showing enamel with amelogenesis imperfecta. (100x magnification and potential of 1.5 kV).

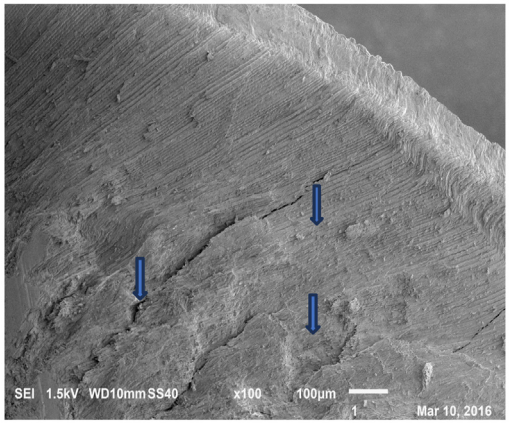

Figure 10. Detailed view of the areas of microfractures in the enamel (arrows). (100x magnification and potential of 1.5 kV).

dental crown was pulverized and stored for further quantitative analysis. SEM revealed more details of the microfractures contained in the enamel of teeth with AI (Figures 8 and 9). A tortuous change in the orientation of enamel prisms can also be seen in some regions. No enamel microfractures were observed in the region of the cervical third. No alteration was identified in the control teeth (Figure 10).

DISCUSSION

Teeth affected by hypocalcified AI have soft, normal-thick enamel that breaks and wears away easily.[6] Furthermore, enamel has a radiodensity similar to that of dentin.[15] The patient reported in this article was an adult male who had significant changes in enamel. The teeth exhibited standard anatomy but broke easily when in normal function, except in the cervical third. Teeth color ranged from light brown to black in the occlusal and middle thirds. On the other hand, in the cervical third, the enamel color was normal. In addition, during anamnesis, the patient reported that two other brothers had the same condition in their teeth. The diagnosis of subtype IIIa hypocalcified AI was established based on the clinical and radiographic characteristics of the teeth and the genetic manifestation pattern.[6] According to Chamarthi et al.,[19] the hypocalcified type is observed in only 7% of cases of AI.

The radiographic appearance of enamel is less opaque than dentin. Enamel thickness is initially normal, but the enamel is soft and easily removed soon after eruption. The microtomography analysis of the teeth revealed that the enamel thickness was apparently normal when compared to the controls. According to Zhang et al.,[20] the thickness of hypocalcified enamel is usually normal when it is not fractured from the underlying dentin during the initial eruption. This indicates that the volume of secreted enamel matrix protein could not be influenced by mutations in FAM83H.

It is believed that a mutation in the FAM83H gene is responsible for the manifestation of this disease subtype.[21,22] Gene expression of the mutation in FAM83H leads to retention of amelogenin.[23] Amelogenins are specific proteins produced by ameloblasts, constitute 90% of the developing enamel matrix and are essential for the development of an enamel layer with normal thickness, architecture, and composition.[24] According to the literature, the mechanisms by which amelogenin direct the growth of enamel crystals are still not fully understood. Apparently, these proteins organize into nanospheres that control the size and orientation of the enamel crystallite by preventing lateral fusion. In this way, they control the mineral deposition on the sides of the crystals.[25,26]

Panoramic radiography revealed the presence of taurodontism in some teeth. Taurodontism is a dental anomaly characterized by enlargement of the body and pulp chamber of a multirooted tooth, which leads to apical displacement of the pulp floor and root bifurcation.[6] There is no record in the literature of cases of taurodontism associated with subtype IIIA

hypocalcified AI. However, this shape anomaly is seen in AI in its hypomature-hypoplastic (IVA) and hypoplastic-hypomature (IVB) forms. It is noteworthy that the patient in this case did not have the necessary characteristics to fit into these two subtypes.

Images obtained by micro-CT and SEM showed the presence of microfractures in the enamel structure of teeth with hypocalcified AI. These defects in the crystalline structure favor enamel breakdown when subjected to masticatory forces. This hypothesis is supported by the clinical characteristics observed in the patient, since all the posterior teeth and the canines had already lost practically all the enamel, as a result of chewing alone. These findings differ from the study carried out by Sa et al.[27] These authors performed an analysis of teeth with hypomatured AI using microtomography. MicroCT showed that the hypomatured enamel layer was thinner and had more irregular surfaces when compared to normal teeth. On the other hand, the presence of pores on the enamel surface compromised by hypocalcified amelogenesis imperfecta was also observed by Sa et al.[27]

There are few morphological studies of teeth with amelogenesis imperfecta using SEM. In 1989, Bäckman et al.[28] used SEM to evaluate deciduous teeth from patients with AI (hypoplastic and hypomature). The enamel of the teeth with hypoplastic AI presented a surface with regularly distributed depressions and rounded appearance. On the other hand, marked defects were seen on the incisal surfaces of teeth with hypomatured AI. A honeycomb appearance was observed in the deeper region of these defects. However, our results corroborate the findings of Zhang et al.[20] These authors also analyzed teeth with hypocalcified AI by SEM and observed that the enamel layer in the affected teeth also showed microfractures. Furthermore, there was evidence that the enamel prisms close to the dentinal-enamel junction were irregular and broken.

The hardness of normal enamel is greater than the hardness of enamel affected by hypocalcified amelogenesis imperfecta.[29] On the other hand, dentin hardness did not differ between normal teeth and those affected by hypocalcified AI. Enamel and dentin hardness were similar for teeth affected by hypocalcified AI. Furthermore, a positive linear relationship was observed between enamel hardness and bond strength.

The patient's treatment included removal of impacted third molars, endodontic treatment of posterior teeth and prosthetic rehabilitation. According to the literature, the combination of surgical procedures to increase the clinical crown, the precise establishment of the vertical dimension of definitive occlusion, correct prosthetic preparations and the technical reconstruction of the dental structures makes it possible for the patient to obtain good aesthetics and normal masticatory function.[16]

MicroCT has been used by biology for the structural evaluation of many objects. In dentistry, the main application of X-ray microtomography has been in the structural analysis of bones and teeth.[19] There is no study in the literature that evaluated teeth with amelogenesis imperfecta using microCT. Although we only used three teeth, they were of a specific type of AI.

CONCLUSION

Amelogenesis imperfecta is a rare genetic anomaly that over time significantly weakens the masticatory function of the affected individual. Detailed knowledge of the condition is essential for its correct diagnosis and treatment. The present study demonstrated that dental enamel in subtype IIIA of the disease exhibits unique characteristics from the microscopic and microtomographic point of view. Furthermore, the possibility of simultaneous occurrence of taurodontism cannot be ruled out.

ETHICAL APPROVAL

Informed consent was taken from patient for publishing of data.

CONFLICT OF INTEREST

The authors declare no conflicts of interest, financial or otherwise related to this case report.

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