

ORIGINAL RESEARCH

Crystalline analysis of dental enamel by X-Ray diffraction on pediatric patients with chronic kidney disease

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Abstract

This study aimed to analyze the crystalline structure of dental enamel in pediatric patients with chronic kidney disease (CKD) by X-ray diffraction (XRD). The six tested samples had a mineral composition similar to hydroxyapatite, according to sheet JCPDS (Joint Committee on Powder Diffraction Standards) card #09-0432, which is normally found in dentine, and presented a lower amount of whitlockites (Ca, Mg)₃(PO₄)₂. Pattern phases showed an increase in organic matter and a decrease in inorganic matter. At an interval of approximately $2\theta = 15.7^\circ$ to 27.2° , amorphous organic matter corresponding to hydrated glucose was found. The hydroxyapatite patterns in this study differed from that of dental enamel found on permanent teeth.

Keywords

Kidney disease; Enamel defects; X-ray diffraction-analysis

1. Introduction

According to the National Kidney Foundation (NKF), chronic kidney disease (CKD) is a chronic condition in which the kidneys are permanently damaged, and kidney function is decreased and worsens over time. CKD can affect people of all ages and race. Children have a lower prevalence of caries but a higher prevalence of calculus, debris, gingivitis and enamel hypoplasia in both permanent and primary dentition [1]. In addition, changes in the development of enamel, oral inflammation, mucosal lesions, reduced saliva flow or xerostomia. A previous study found no significant difference in daily tooth brushing frequency and periodic dental check-up frequency [2]. Moreover, children with nephrotic syndrome and CKD are often associated with tooth-related disorders, persistent deciduous teeth and impacted teeth, abnormal crown or root shape, developmental defects of the enamel, pulp stones and bone structure disorders [3].

The effects of CKD on pediatric patients can alter their quality of life because it can lead to disturbed oral functions and psychological and emotional issues [4]. Epidemiological studies estimate that there are 85 cases per million people in the population (pmitp) in the USA and 62 cases pmitp in Europe [5].

There is a high risk of developing oral manifestations in both hard and soft tissues, either due to the disease itself or because of the medications used [6, 7]. Enamel defects are one of the main oral manifestations in patients with CKD [8–10]. The prevalence of these defects in Mexico was reported to be about 7.5% on permanent teeth and 10.0% on primary teeth [11].

Environmental and genetic influences can alter enamel de-

velopment. Short-duration environmental stressors can lead to localized defects, whereas chronic stressors, such as systemic diseases, vitamin deficiencies and/or medications, are associated with generalized defects [12, 13]. Since ameloblasts were found to be very sensitive to any alteration and incapable of regeneration after receiving damage [14–16]. It is important to note that not all teeth and surfaces are equally affected by these defects, even if they develop simultaneously [12].

The phenotype of the resulting enamel (hypomineralization, hypoplasia and/or hypomaturation) during the different stages of histodifferentiation, apposition and mineralization during amelogenesis varies according to the type of stress and the intensity of the provoking stimulus [17, 18].

Criteria for the clinical evaluation of enamel defects were proposed to categorize them based on a severity grade [19–22]. Also, complex microstructure and dental enamel properties have been studied using various qualitative and quantitative techniques to better comprehend physical and chemical processes associated with the formation and destruction of biological apatite [23–25]. Hence, it is of great importance to investigate the crystalline structure of pediatric patients with CKD.

2. Materials and method

2.1 Participants

Patients diagnosed with kidney disease and primary or mixed dentition from the Department of Pediatric Nephrology at the Fray Antonio Alcalde Hospital were included in this study. All patients have no medical history and were men under 10 years

of age.

2.2 Procedure

Sampling type: Non-probabilistic for convenience. Six samples meeting the criteria were selected for analysis. Each dental organ was subjected to cleaning (brushed with tap water) and conservation (saline solution). Although the vestibular face is where dental enamel defects occur most frequently, cuts were made with a diamond disc at low speed in the transverse direction to eliminate the remnant of the resorbed roots and analyze the entire surface of the teeth based on pathology (Fig. 1) so that they could be entered into the XRD beam (Fig. 2).

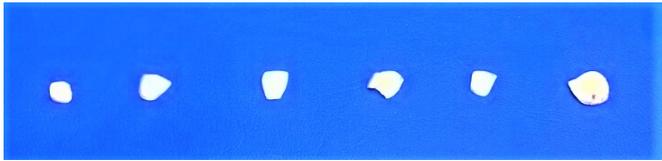


FIGURE 1. Analysis of the six samples and pathology of the entire surface of the enamel.

2.3 Analysis strategy

An Panalytical model Empyrean (Netherlands) Diffractometer (XRD) at the Laboratory of Physics from the University Center of Exact Sciences and Engineering was used. Measurements were taken using the coupled scan on region 10° – 90° at 2θ , using a pass length of 0.026 and a constant measurement of 30 seconds per pace. Phase identification was performed using the PDF-4+ 2019 RDP Data base, taken from the International Center of Diffraction Database (ICDD, Pennsylvania, PA, USA).

3. Results

The samples showed the presence of more amorphous than crystalline matter. However, in most cases of diffraction patterns, some peaks located at $2\theta = 25.9^{\circ}$, 31.8° , 32.3° , 33.0° , 34.0° , 39.8° , 46.7° and 49.5° stood out. Based on the sheet JCPDS (Joint Committee on Powder Diffraction Standards) #09-0432, this corresponded to hydroxyapatite (Fig. 3, Sample 1).

To identify some of the peaks located on the $2\theta = 15.7^{\circ}$ to 27.2° region, the diffraction sheet JCPDS #001-0330 was used. This peak corresponded to alpha-D-glucose monohydrate. The peaks associated to this material were found at $2\theta = 12.6^{\circ}$, 19.6° , 20.6° , 23.0° , 25.4° , 27.8° , 28.8° , 31.3° and 35° (Fig. 3, Sample 2).

It is worth mentioning that other diffraction sheets associated with other monosaccharides were used, such as alpha-D-galactose (JCPDS card #029-1719), beta-D-galactose (JCPDS card #029-1718), beta-D-fructose (JCPDS card #029-1717) and hydrated glucose (JCPDS card #002-0224). However, the positions of the alpha-D-glucose monohydrate peaks presented a higher peak concordance with the analyzed samples, especially at the $2\theta = 15.7^{\circ}$ to 27.2° region (Fig. 3, Sample 3).

Based on these results, we could affirm that a major part of the amorphous material localized at the $2\theta = 12.6^{\circ}$ to 31.3° region could be associated with alpha-D-glucose monohydrate (Fig. 3, Sample 4–6).

4. Discussion

The mineral phase of enamel identified corresponds to a calcium phosphate with a $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ apatite structure [26, 27]. However, taking stoichiometry and the association with various elements found in the Ca-P hydroxyapatite (Hap) into consideration, it was inferred as $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, the $\text{Ca}_{10}(\text{PO}_4)_5(\text{CO}_3)(\text{OH})_2$ and $\text{Ca}_5(\text{PO}_4)_3(\text{OH}, \text{Cl}, \text{F})$.

In healthy teeth, enamel and dentine crystallinity is higher [22]. In addition, the hydroxyapatite in the enamel was found to have a more regular pattern. Further, the diffraction pattern peaks for the enamel were more intense [25], especially between the lines at the $2\theta = 25^{\circ}$ to 35° interval. Comparatively, the dentine phases were irregular and undefined.

A previous study [28] reported on the changes in both the amount and proportion of elements in samples with hypoplastic lesions in deciduous teeth. Another study [29] found that calcium apatite in permanent hypomineralized organs was the only phase of calcium phosphate present on the hypomineralized enamel.

Systemic alterations of urea, potassium and other elements in pediatric CKD patients may alter the amelogenesis process in primary teeth, specifically during the mineralization process, where the processes for these routes are interrupted [12].

A shift for one of the intensity peaks in the crystalline phase was detected in the enamel between the $2\theta = 32^{\circ}$ to 35° interval. Other groups described this phenomenon to be due to the additional incorporation of carbonate, sodium and magnesium into the apatite structure, which changed the crystalline structures and led to the shifting of one or more peaks.

Over 60% of primary teeth demonstrated an aprismatic surface with a width of 16–45 μm [30], also known as the Darling zone. This border is permeable to the entry of bacterial products, especially acids, and has a rough surface that favors dental biofilm retention and subsequent demineralization [31].

The presence of organic matter coincides with the results of a previous work [32], in which it was reported that the organic matrix of dental enamel was unorganized at the molecular level, along with a lack of detectable orientation on XRD patterns.

Reduced calcium, phosphorous or vitamin D levels caused by CKD might also negatively impact enamel development [1, 33].

5. Conclusions

The analyzed samples showed enamel defects clinically manifested as opacities, discontinuations and white patches on the adamantine surface, which concurred with previous studies as these morphological characteristics could be seen in a mineral phase, similar to dentine due to the absence or quality defects of inorganic material.

Lastly, the presence of glucose in the diffraction pattern of

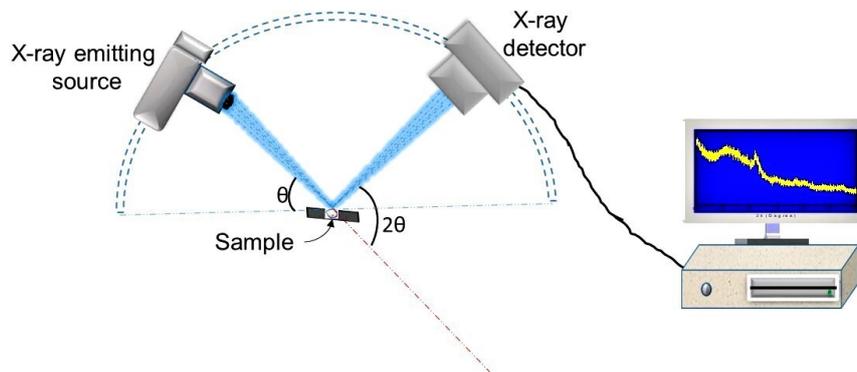


FIGURE 2. Diagram illustrating how the X-rays were projected on the surface of the dental piece and the diffraction pattern generated by the detector to obtain the peaks height corresponding to the intensity of the impacting radiation.

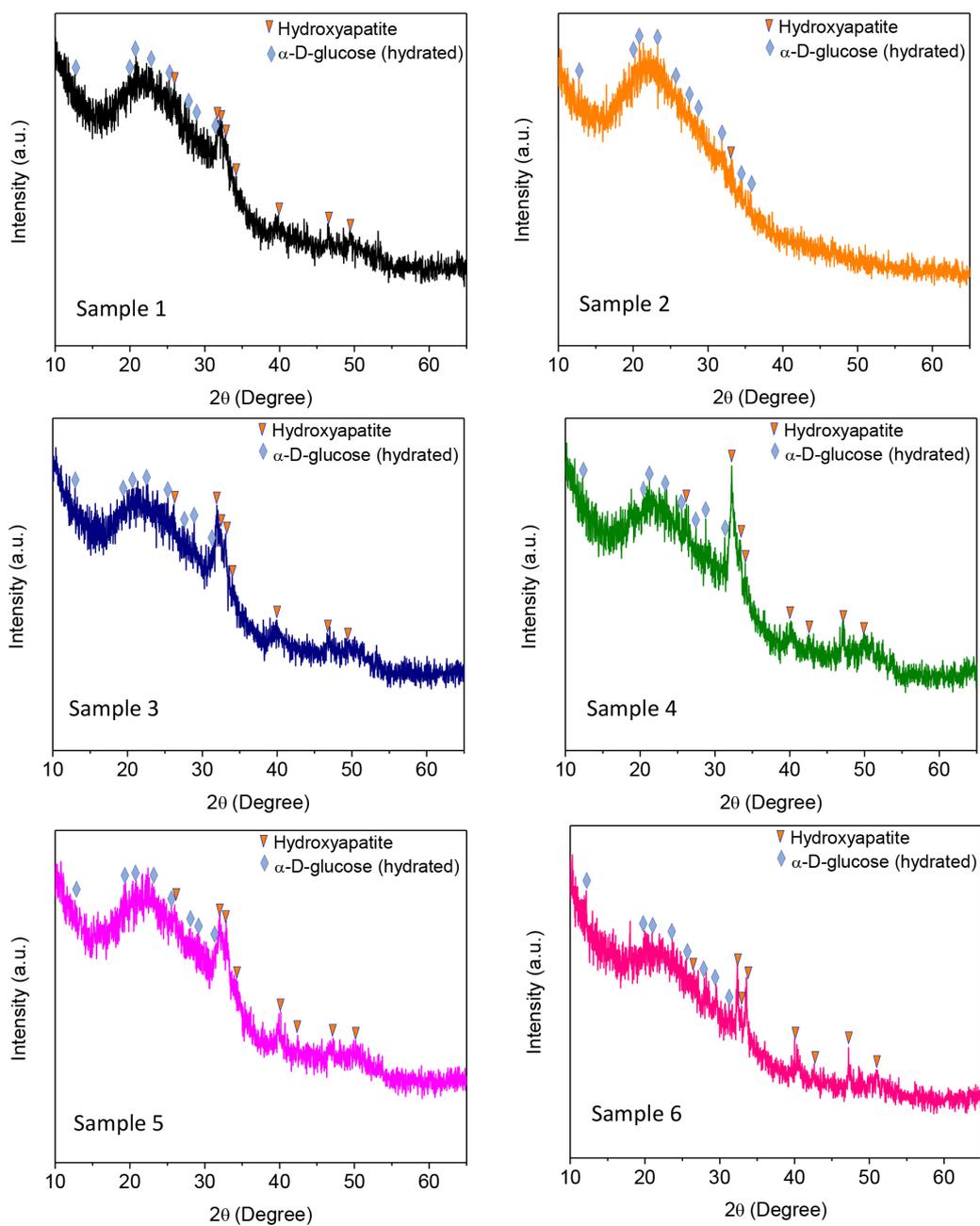


FIGURE 3. XRD patterns of the dental piece with dental enamel.

the enamel as an organic layer (Fig. 3) in the outer surface zone might have inherently impacted renal metabolic problems.

AVAILABILITY OF DATA AND MATERIALS

The data presented in this study are available on reasonable request from the corresponding author.

AUTHOR CONTRIBUTIONS

ICA—Conceptualization, Validation, Supervision, Resources Writing Review Editing, Visualization. MIHR—Conceptualization, Supervision, Writing Original draft preparation. MALA—Formal Analysis Application, Data curation, Software. CCAS—Project-administration, Funding acquisition.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All procedures performed in studies involving human participants were in accordance with the Ethics Committee of the Dental School and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study details were explained to the parents, and their signed informed consent was obtained.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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