Regional Odontodysplasia: Morphological, Ultrastructural, and Immunohistochemical Features of the Affected Teeth, Connective Tissue, and Odontogenic Remnants

Roman Carlos, DDS Elisa Contreras-Vidaurre, DDS, PhD
Oslei Paes de Almeida, DDS, PhD Kenia Regina Silva, DDS, MSc
Patrícia Gonçalves Abrahão, DDS, MSc
Águida Maria Menezes Aguiar Miranda, DDS, MSc
Fábio Ramôa Pires, DDS, PhD

ABSTRACT

Purpose: Regional odontodysplasia (**RO**) is a rare developmental odontogenic defect. The affected teeth are described as "ghost teeth," due to malformation of dentin, enamel, and cementum. The purpose of this study was to describe the ultrastructural characteristics of the affected teeth as well as the immunohistochemical features of the soft tissue associated with 2 cases of regional odontodysplasia.

Methods: Two cases diagnosed as RO were included on the study. After diagnosis, affected teeth and associated soft tissue were surgically removed from both patients and processed for light and scanning electron microscopy (SEM) analysis. For light microscopy, the specimens were decalcified in 5% nitric acid for 10 to 16 days and stained with hematoxylin and eosin. For SEM studies, specimens were cleaned with diamond paste and, after polishing, treated with 37% phosphoric acid for 5 minutes and covered by a thin layer of metallic gold. Soft tissues covering and surrounding the extracted teeth were routinely processed, and submitted to immunohistochemical reactions against pan-cytokeratins, cytokeratin 19, vimentin, laminin and collagen IV.

Results: Both enamel and dentin showed gross alterations, including hypomineralization and discoloration, being more prominent on the coronal structures than the radicular structures. Ultrastructural features included alterations on the prismatic enamel surface, dentinal grooves, interglobular dentin, and hypoplastic and hypocalcified dental hard tissues. The soft gingival and alveolar tissue surrounding and covering the affected teeth showed calcifications and odontogenic remnants, positive for cytokeratin 19, laminin, and collagen IV.

Conclusions: Enamel and dentin are grossly altered in regional odontodysplasia, while cementum is less affected, and soft-tissue calcifications are associated with odontogenic cytokeratin-positive epithelial remnants, in addition to mesenchymal components.

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KEYWORDS: REGIONAL ODONTODYSPLASIA, GHOST TEETH, ODONTOGENIC REMNANTS

Dr. Carlos is Chairman, Department of Pathology, and Dr. Contreras-Vidaurre is Chairman, Department of Oral Medicine, both in the Centro Clínico de Cabeza y Cuello, Guatemala City, Guatemala; Dr. Silva and Dr. Abrahão are graduated students, and Dr. Almeida is Chairman, all in the Department of Oral Pathology, Piracicaba Dental School, State University of Campinas, Piracicaba, Brazil; and Dr. Miranda and Dr. Pires are Professors, both in the Department of Stomatology, School of Dentistry, Estácio de Sá University, Rio de Janeiro, Brazil.

Correspond with Dr. Pires at ramoafop@yahoo.com

Regional odontodysplasia (**RO**) is a rare developmental dental defect involving both the ectodermic and ectomesenchymal-derived structures (enamel, dentin, cement, and pulp).[1-5] It usually affects groups of primary and permanent contiguous teeth and has been reported under a variety of terms, but RO is the most common used terminology.[3-5] It is typically seen isolated, but eventually it can be associated with some developmental

disorders. These can include hypophosphatasia, unilateral facial hypoplasia, neurofibromatosis, vascular nevi, mental impairment, and ectodermal dysplasia, as well as epidermal nevus, tricho-dental-osseous, Papillon-Lefèvre, and Gorlin syndromes.[3,6]

Diagnosis of RO is based on clinical and radiographic characteristics of the affected teeth. Treatment frequently includes tooth extraction followed by prosthetic rehabilitation, and most RO reports have focused on its clinical and treatment features.[3] The purpose of this study was to report the macroscopic, microscopic, immunohistochemical, and ultrastructural characteristics of the affected teeth and adjacent soft tissue on 2 cases of regional odontodysplasia.

METHODS

Two patients diagnosed as having RO, based on clinical and radiographic features, were included in the study. After diagnosis, both patients were managed through removal of the affected teeth and associated soft tissue under local anesthesia and prosthetic rehabilitation.

Teeth removed from both patients were longitudinally sectioned and processed for light and scanning electron microscopy (**SEM**) analysis. For light microscopy, the specimens were decalcified in 5% nitric acid for 10 to 16 days and stained with hematoxylin and eosin. For SEM (Jeol, JSM, 5600SLV, Jeol, Tokyo, Japan) studies, the specimens were cleaned with diamond paste and, after polishing, immediately ultrasonicated in water for 5 minutes. The specimens were then treated with 37% phosphoric acid for 5 minutes and covered by a thin layer of metallic gold.

Gingival and alveolar soft tissues covering and surrounding the extracted teeth were routinely processed, and 5-µm sections were stained with hematoxylin and eosin. For immunohistochemical reactions, 3-µm sections were stained with antibodies against pan cytokeratin (clone AE1/AE3, Dako, Carpinteria, US, dilution 1:500), cytokeratin 19 (clone CCK108, Dako, Carpinteria, US, dilution 1:400), vimentin (clone Vim3B4, Dako, Carpinteria, US, dilution 1:400), laminin (clone 4C7, Dako, Carpinteria, US, dilution 1:50), and collagen IV (clone CIV22, Dako, Carpinteria, US, dilution 1:10).

After deparaffinization and incubation for 30 minutes in 1% hydrogen peroxide solution, the specimens were submitted to microwave antigen retrieval using a citrate buffer solution (10 mm, pH 6.0) for 20 minutes. Specimens were incubated with primary antibodies for 16 hours at 4°C and then with secondary antibodies (dilution=1:500) for 30 minutes. After exposition to streptavidin-biotin complex (ABC, Dakopatts, Glostrup, Denmark; dilution=1:500) for 30 minutes, diaminobenzidine (DAB, Dako, Carpinteria, US) was used as the chromogen and hematoxylin as the counterstaining.

RESULTS

CLINICAL AND RADIOGRAPHIC FEATURES: CASE 1

A 12-year-old female complaining about the failure to erupt of several permanent maxillary teeth was referred for evaluation. Her mother reported that the primary teeth of the region were surgically removed some years ago, but the permanent teeth never erupted. She also reported that the primary teeth were clinically normal and the general health of the girl was otherwise normal. Clinical examination revealed a swelling on the permanent maxillary right alveolar mucosa, extending from the maxillary left central incisor to the maxillary right first molar, and absence of all teeth in the region (Figure 1). Panoramic and periapical radiographs showed unerupted teeth in the region, all of them with incompletely formed with short roots, wide pulp chambers, and thin hypocalcified enamel and dentin layers (Figure 2). A diagnosis of RO was made, all affected teeth were surgically removed, and the patient received prosthetic rehabilitation.

CLINICAL AND RADIOGRAPHIC FEATURES: CASE 2

A 25-year-old male was referred for evaluation complaining of fragile and fractured teeth since childhood. The patient reported that primary and permanent teeth on the anterior mandible were very fragile and brown-colored, since they

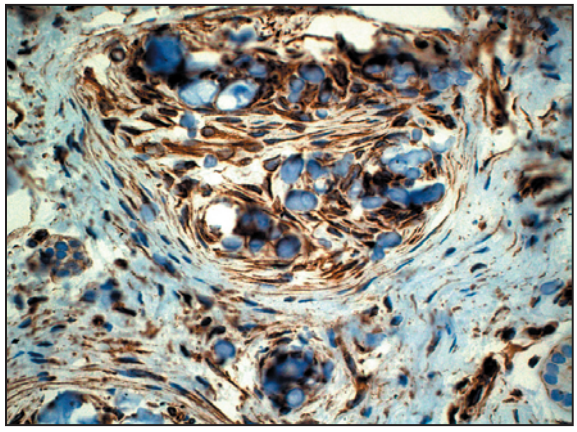

Figure 1. Clinical aspect of regional odontodysplasia (case 1) showing the missing teeth area and a swelling on the anterior maxillary region.

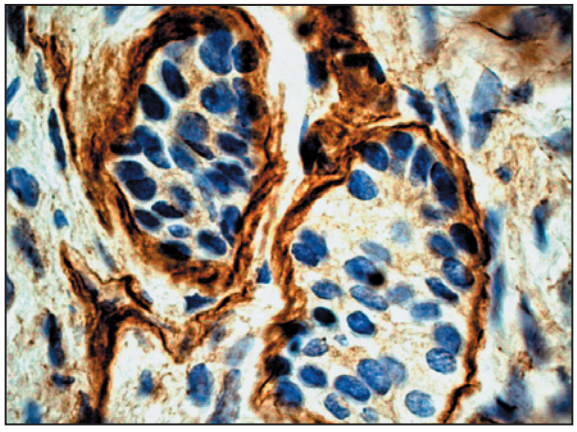

Figure 2. Panoramic radiograph of regional odontodysplasia (case 1) showing the "ghost teeth" in the anterior maxillary region.

erupted on the oral cavity. Medical history was noncontributory. Physical examination revealed multiple microdontic and irregularly shaped brown teeth with fractured crowns extending from the right lower canine to the left first molar (Figure 3). Some teeth were missing in the area and others were previously treated endodontically. Panoramic and periapical radiographs showed short teeth with incomplete root formation, wide pulp chambers, and thin hypocalcified enamel and dentin (Figure 4). The diagnosis was RO, and the patient was referred for surgical removal of the affected teeth followed by prosthetic rehabilitation.

MACROSCOPIC, LIGHT MICROSCOPIC, ULTRASTRUCTURAL, AND IMMUNOHISTOCHEMICAL FEATURES

Macroscopically, the affected teeth showed a yellowish-brown color, irregular morphology, and extensive defects of enamel mineralization, with focal areas of dentin exposure. After decalcification, most of the remaining enamel was lost, but the residual areas showed grossly altered enamel in quantity and distribution. SEM observations performed on two teeth from each of the cases, revealed that the enamel was generally thin, the direction of enamel prisms was irregular, and the enamel surface was not uniform in appearance (Figure 5). Numerous small depressions were related to prism cores. Hypoplastic areas showed a thin layer of poorly organized enamel in the outermost surface.

In general, coronal dentin was more affected than radicular dentin. Dentin was thin and globular, with interglobular spaces and cellular and amorphous areas. Predentin was prominent in some regions. SEM of dentinal tubules showed alterations of orientation, density, size, and shape (Figure 6). Clefts of various sizes could be observed in many areas of the coronal dentin, giving a fibrous aspect. We did not observe any abnormalities in the dentin-enamel junction. Cementum, like enamel and dentine, also was thinner than normal, however, it showed no evident structural changes (Figures 7 and 8). Some scalloping of the cement surface was observed in isolated areas. The pulp chamber was larger than normal, showing many calcifications—some attached to the dentin wall.

Soft tissue from the gingival/alveolar mucosa was composed by dense collagenous fibrous tissue, with focal aggregations of calcified bodies and islands of

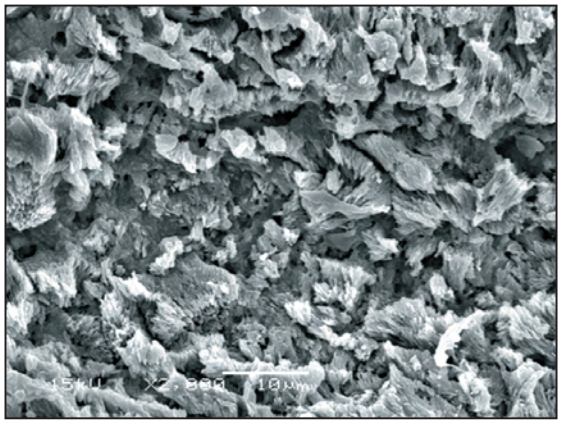

Figure 3. Clinical aspect of regional odontodysplasia (case 2), showing multiple microdontic teeth in the anterior mandibular region.

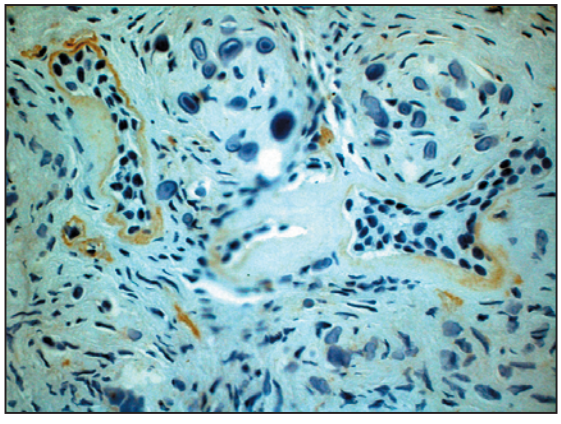

Figure 4. Periapical radiographs of the anterior mandibular region of regional odontodysplasia (case 2), showing "ghost teeth" with shortening of teeth, incomplete root formation, wide pulp chambers and thin hypocalcified enamel and dentin.

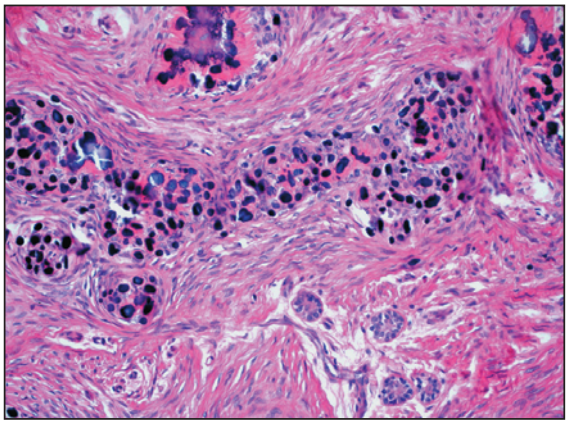

Figure 5. Ultrastructural aspect of the enamel from a tooth affected by regional odontodysplasia showing irregular oriented enamel prisms and numerous small depressions related to prism cores (scanning electronic microscope, X2,000).

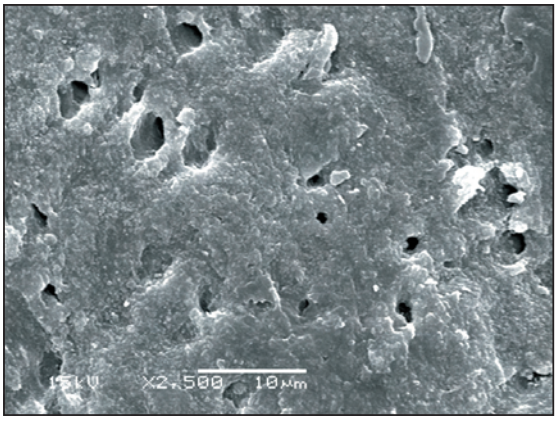

Figure 6. Scanning electron micrograph of coronal dentin showing alterations of orientation, density, size, shape, and distribution of the dentinal tubules (scanning electronic microscope, X2,500).

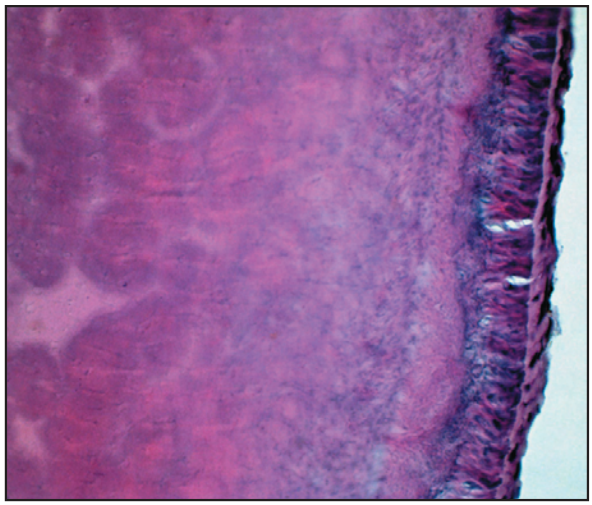

Figure 7. Scanning electron micrograph from a tooth affected by regional odontodysplasia showing a thinner but otherwise morphologic normal cementum (scanning electronic microscope, X200).

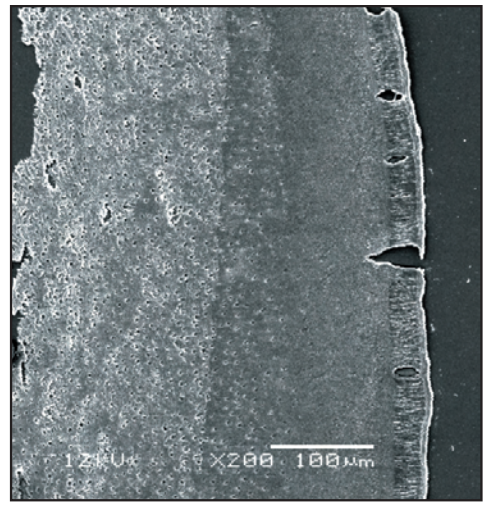

Figure 8. Globular dentin and irregular dentinal calcifications in a tooth affected by regional odontodysplasia (hematoxylin and eosin, X50).

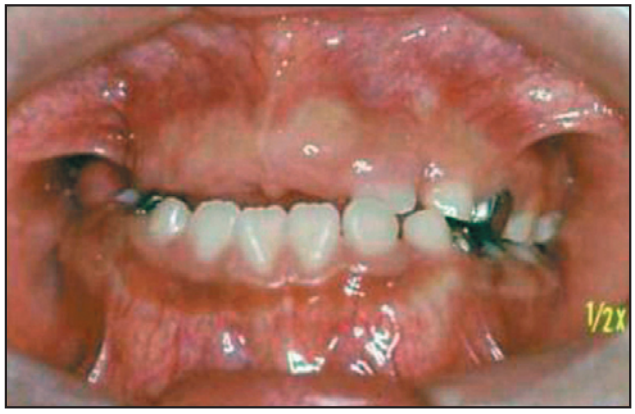

Figure 9. Gingival tissue from an area affected by regional odontodysplasia showing an extensive area of calcification in the connective tissue associated with epithelial islands (hematoxylin and eosin, X200).

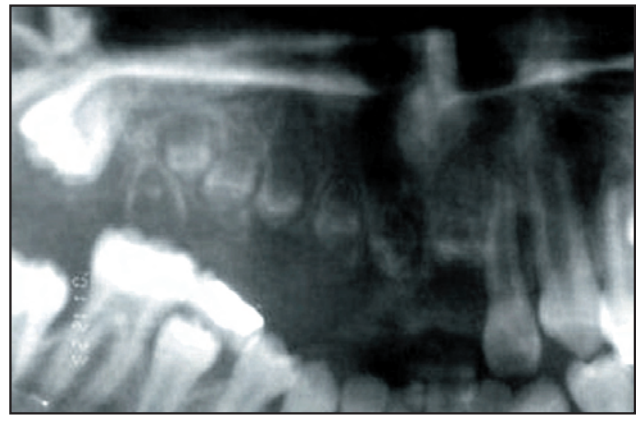

Figure 10. Gingival tissue from an area affected by regional odontodysplasia showing that most of the calcifications are involved by connective tissue fibers and fibroblasts (immunoperoxidase, vimentin, X200).

odontogenic epithelium (Figure 9). Small round calcifications were abundant, coalescing in some areas to form larger calcified bodies. An extracellular matrix lined the larger calcifications, while a dense hyaline membrane surrounded the islands of odontogenic epithelium. Superficial mucosal areas presented variable degrees of chronic inflammatory infiltrate.

Immunohistochemical analysis of the soft tissue showed that odontogenic remnants were positive for pan cytokeratin and cytokeratin 19 and that the ectomesenchymal tissue was positive for vimentin (Figure 10). Cells associated with calcifications were mainly positive for vimentin, but some also expressed pan cytokeratin and cytokeratin 19. Basal lamina involving the epithelial odontogenic islands was strongly positive for laminin (Figure 11), but only lightly positive for collagen IV. Some epithelial islands showed a thick hyaline matrix on the periphery, which was very lightly positive for collagen IV in some regions (Figure 12).

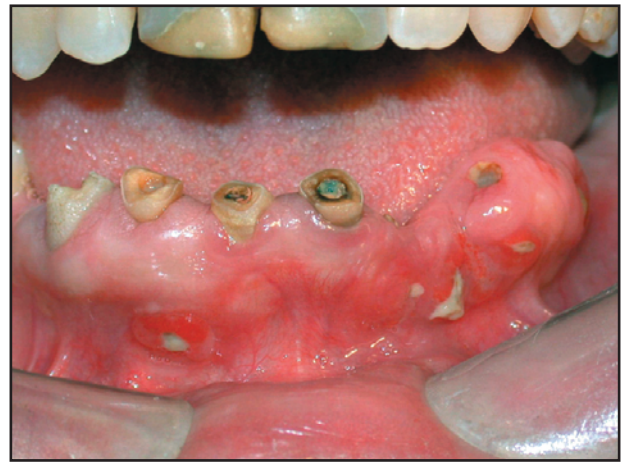

Figure 11. Island of odontogenic epithelium from an area affected by regional odontodysplasia showing positivity to laminin in the basal membrane (immunoperoxidase, laminin, X400).

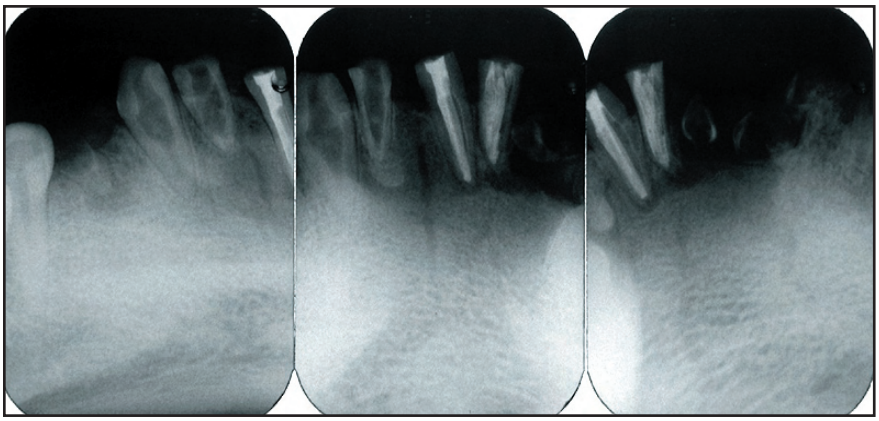

Figure 12. Hyaline matrix lightly expressing collagen IV, involving an odontogenic epithelial island from an area affected by regional odontodysplasia (immunoperoxidase, collagen IV, X200).

DISCUSSION

RO is a rare developmental anomaly which involves the odontogenic components derived from both epithelium and connective tissue. According to Crawford and Aldred,[3] diagnosis of RO is predominantly clinical and radiographic, but its histopathological features are also characteristic. The etiology of RO is not well understood, and several possibilities have been reported in the literature, including local circulatory disorders, trauma, latent viral infection on tooth germs, metabolic disorders, neural alterations, genetic transmission, other local infections, irradiation, and somatic mutations.[3,5-8] The most accepted etiology for RO is an alteration on the vascularization of the affected area, leading to disturbances on tooth development. In fact, some cases of RO have been associated with vascular nevi in the area of the affected teeth.[9-11] As most reported cases, we could not identify any local or systemic etiologic factors in our cases of RO.

RO is more common in the maxilla than in the mandible, usually affecting one quadrant and commonly associated with delayed or absent teeth eruption.[4,9,12] Although most cases are unilateral and restricted by the midline, some can affect more than one quadrant crossing the midline or more than one noncontiguous quadrant, as shown in case 2 of this study.[10-13] Anterior teeth are more affected, with a slight female predilection, and patients are usually diagnosed within the first 2 decades of life without racial preference.[3,4,9] The teeth of the affected area are usually microdontic and not well-formed, showing brown to yellow discoloration, coronal and radicular dilacerations, fractures, and caries—with a fragile hard tissue structure reflecting the several degrees of enamel and dentin hypoplasia.[1,4] The fragility and irregular surface of the affected teeth can lead to the development of pulp and periodontal pathosis.[9] The dental literature reports an age range from 4 to 23 years,[4] but one of our patients was a 25-year-old male with both primary and permanent teeth affected by the condition. This is an uncommon feature, since RO frequently affects both dentitions, and diagnosis is usually established during eruption of the primary or mixed dentition.[3,5,9] When there are no more primary teeth in the affected area, previous history should be carefully obtained to confirm the severity and spectrum of the disease.

Radiographically, the affected teeth described in our 2 cases showed the classic features of RO, including short roots, wide pulp chambers, and large apical foramens. There was a marked reduction on enamel and dentin thickness and radiographic density, with no distinct enamel-dentin limit, giving the classical appearance of "ghost teeth."[1,4,5,12,14,15] Gerlach et al[13] followed an RO patient for 6 years and reported continuous dentin formation in the affected "ghost teeth," reduction of the pulpal chamber, and relative normalization of the radicular shape. Nevertheless, this does not seem to be the usual course, depending upon the severity of the teeth alterations, and is limited by the functional and esthetic possibility of maintaining the teeth on the area.

Macroscopically, the involved teeth presented an irregular morphology with rough surface and yellowish to brownish discoloration. Few papers in the literature have focused on the scanning (**SEM**) and transmission electron microscopic (**TEM**) aspects of RO.[15-21] Enamel and dentin showed extensive gross mineralization defects through SEM. Enamel was thin, hypoplastic, hypocalcified, and poorly organized.[1,22,23] Enamel prisms were irregularly oriented, with evident alterations in the hydroxyapatite crystals and interprismatic regions, giving a laminated appearance to the enamel. Defects of morphology and mineralization of the enamel crystals, leading to large defects have also been demonstrated by TEM.[21] Areas of hypocalcified and hypoplastic enamel could eventually be seen juxtaposed to relatively normal enamel, suggesting that there was an interruption of normal ameloblastic function in a specific period of odontogenesis.[16]

Dentin and pulp were also grossly altered. Coronal dentin showed several grooves, interglobular dentin, amorphous material, and some areas of hypercalcification.[15,17,18,23] The grooves have been reported as extensions of the enamel-dentine junction to the pulp, leading to free access to pulpal tissue and, consequently, infection. They can be formed by a dentin matrix lacking collagen fibers, and sometimes this irregular dentin can resemble osteodentin.[19] We did not observe any abnormalities in the dentin-enamel junction, as previously reported.[24,25] Amorphous areas of dentin seem to be composed mostly of glycosaminoglycans, with no collagen proteins, and they are considered more mineralized than normal dentin.[23] Dentin tubules were reduced in number, sclerotic, and irregularly distributed and oriented, showing different shapes and sizes.

In all the affected teeth of this study's 2 cases, radicular dentin was less affected than coronal. We found areas

presenting a wide zone of predentin mixed with areas missing predentin. The pulp showed laminated and perivascular calcifications, as previously reported.[5,16] Some authors consider that cementum can be normal in RO.[8,22,24-27] In our cases, it was thinner than normal, but without evident structural changes—except for some scalloping of the cement surface in isolated areas.

Soft tissue covering the affected teeth typically presents small rounded or irregular foci of calcification, as well as islands of odontogenic epithelial remnants.[5,6,13,16-18] Both cases reported here showed prominent calcifications on the soft tissue. Most of them were associated with vimentin-positive cells, but it is interesting to report that some areas of calcification were associated with epithelial odontogenic remnants, as shown by the immunohistochemical positivity to cytokeratins. Therefore, as previously suggested, some of these calcifications can result from degenerative changes of the odontogenic epithelial islands. In fact, epithelial cells commonly show vacuolization that could lead to degeneration and eventual calcification.[19] Matrix metalloproteinases and their natural inhibitors play an important role in the breakdown of collagen, and it has been considered that they participate in the mechanism of calcification seen in RO.[3] Some soft tissue calcifications in RO, however, have been also found in association with microfibrils, different from collagen fibers.[16,18] It is important to differentiate calcifications seen in RO from those eventually found in hyperplastic dental follicles, with the latter being associated with normal enamel and dentin.[20] Epithelial cells juxtaposed to some of the calcified bodies clearly indicates an association between odontogenic epithelium and the calcifications.

Conventional RO management includes teeth removal with subsequent insertion of removable prosthetic appliances, followed by fixed prosthetic rehabilitation, dental implants, or even teeth autotransplantation when desirable and indicated.[3,9,12,21,26] Eruption of affected teeth usually reveals fragile, dark-colored, caries-prone teeth that are not usually able to be maintained on the oral environment due to functional and esthetic limitations. Continuous mineralization of the affected teeth has been reported in the dental literature,[13] however, and conservative dental and orthodontic treatments have been reported as adjunctive measures in selected cases, especially when dealing with erupted affected teeth, to avoid early bone resorption.[3,9,12,26]

CONCLUSIONS

Etiology and pathogenesis of regional odontodysplasia is still unknown, and a better understanding of the morphological characteristics of the involved teeth, as presented here, MAY help clarify these developmental tooth defects. Our results showed that enamel and dentin are grossly altered in RO, while cementum is less affected. In addition, soft-tissue calcifications seen quite commonly in RO are associated with mesenchymal vimentin-positive cells and with odontogenic cytokeratin-positive epithelial remnants.

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