Demarcated Developmental Enamel Defects in Cleft-Adjacent Tooth

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Background

Developmental enamel defects, including demarcated hypomineralized opacities, frequently affect children with cleft palate and are phenotypically similar to molar-incisor hypomineralization (MIH). Clinically, these defects present as well-demarcated opacities adjacent to sound enamel, ranging from chalky white to dark brown discolorations (Weerheijim et al., 2001. Hypomineralized enamel exhibits reduced mineral content, increased residual organic matrix, hypersensitivity, and increased susceptibility to post-eruptive breakdown (Inchingolo et al., 2023).

The alveolar cleft region is particularly vulnerable to developmental disturbances affecting tooth formation (Fonseca-Souza et al., 2022). Although several etiologic factors have been proposed, the biological mechanisms underlying hypomineralized enamel defects in remain incompletely understood.

Objective

To characterize and visualize mineral content and enamel matrix protein distribution within demarcated hypomineralized enamel opacities in a cleft-adjacent tooth.

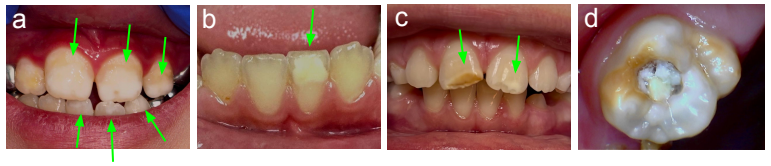

Figure 1. (a) Large demarcated opacities present on tooth #8, #9, #10 with small demarcated opacities on #24, #25, #26. (b) Large demarcated opacity present on tooth #24. (c) Post-eruptive breakdown of incisal edge of tooth #8 with demarcated lesion around perimeter. Small demarcated opacity on tooth #9. (d) Cavitated occlusal caries on tooth #14 with brown demarcated lesion on buccal surface and white demarcated lesion on the occlusal surface.

Methods

An extracted maxillary premolar adjacent to a cleft palate was collected from a pediatric patient at UT Health San Antonio following IRB approval. A maxillary premolar extracted for orthodontic purposes served as a control. Teeth were stored in 10% neutral buffered formalin, rinsed in phosphate-buffered saline, and imaged using microcomputed tomography (microCT; Bruker Skyscan 1172) to assess mineral density and internal opacity distribution. Stereomicroscopy was used to identify and visualize demarcated enamel opacities (Figure 2).

Specimens were then decalcified in 4% EDTA, paraffin embedded, sectioned, and mounted on glass slides. Histologic staining with Masson's trichrome was performed, followed by immunohistochemistry using antibodies targeting ameloblastin (AMBN), amelogenin (AMEL), and kallikrein-related peptidase-4 (KLK4). Staining patterns were evaluated qualitatively using light microscopy and compared between cleft-adjacent and control teeth (Figures 3 and 4).

Results

Clinical examination demonstrated large and small demarcated enamel opacities in teeth adjacent to a cleft, consistent with MIH-like presentations (Figure 2). MicroCT imaging revealed well-demarcated regions of decreased mineral density within the cleft-adjacent premolar that corresponded to the clinically visible opacities (Figure 2).

During EDTA decalcification and processing, enamel from the control tooth was not retained for sectioning. In contrast, a flexible ("leathery") residual enamel fragment from the hypomineralized tooth was preserved and successfully embedded and sectioned. Masson's trichrome staining demonstrated increased organic matrix retention within hypomineralized enamel, with visible Hunter–Schreger banding patterns similar to those observed in normally mineralized enamel (Figure 4).

Immunohistochemical analysis revealed localization of AMBN within enamel rods, rod sheaths, and along the dentinoenamel junction (DEJ), with accentuated retention of AMBN in hypomineralized regions. AMEL and KLK4 localization was observed within rod and interrod enamel (Figure 4). Pronounced and irregular scalloping of the DEJ was observed in the cleft-affected tooth compared with the control tooth (Figure 3). Within hypomineralized zones, alternating rod trajectories consistent with Hunter–Schreger bands were also identified.

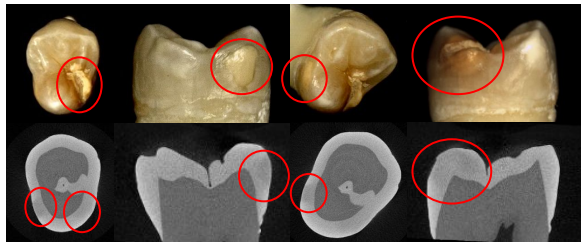

Figure 2. Stereomicroscopy (top row) and MicroCT (bottom row) images of a hypomineralized premolar. Occlusal view (first column), mesial view (second column), mesial-occlusal view (third column), distal view (fourth column).

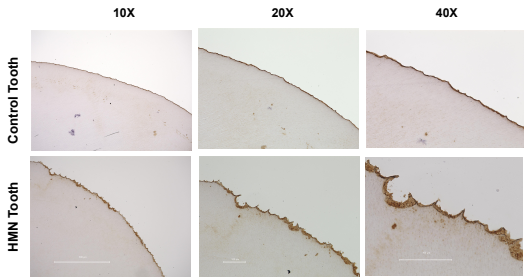

Figure 3. Dentin-enamel Junction (DEJ) scalloping at 10x (first column), 20x (second column), and 40x (third column) magnification. AMBN Immunohistochemical slides taken at level of DEJ of lingual/axial surface of hypomineralized (HMN) premolar (top row) and buccal/axial surface of control premolar (bottom row)

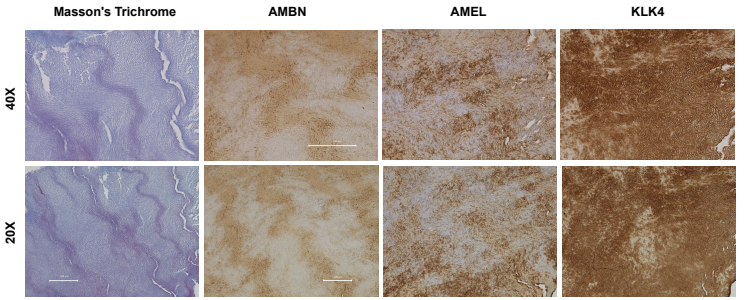

Figure 4. Immunohistochemical and histologic staining of hypomineralized enamel matrix (from residual fragment) at 40x (top row) and 20x (bottom row) magnification. IHC staining: Masson's Trichrome (first column), AMBN (second column), AMEL (third column), KLK4 (fourth column).

Conclusions

- Enamel opacities in cleft-adjacent teeth are hypomineralized and exhibit increased retention of organic matrix.
- Localization and accumulation of enamel matrix proteins within hypomineralized regions suggest impaired protein clearance during the maturation stage of amelogenesis.
- Altered rod structure and pronounced dentinoenamel junction scalloping may reflect disrupted ameloblast function related to local cleft morphology.
- These demarcated defects present clinically similar to MIH and provide biological insight into mechanisms underlying developmental enamel opacities.

Limitations:

- Descriptive analysis limited to a single cleft-adjacent tooth.
- Mineralized enamel is lost during decalcification and cannot be further analyzed histologically

Future Developments

- Inclusion of additional cleft-adjacent hypomineralized teeth.
- Comparative analysis with extracted MIH-affected teeth.
- Expanded immunohistochemical evaluation including enamelin and MMP20

Acknowledgements and References

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