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CASE REPORT

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Incipient ameloblastoma or odontogenic hamartomatous proliferation: is *BRAFV600E* mutation helpful to distinguish them?

Abstract:

The distinction between odontogenic hamartomatous proliferation and other odontogenic cysts and tumors poses a diagnostic challenge. This report presents a unique case of an 18-year-old male who complained of pain and pus discharge in the right posterior mandible for three weeks. Upon intraoral examination, the patient presented with erythema and purulence in the posterior mandible, initially diagnosed as pericoronitis. Computed tomography revealed a well-defined, hypodense, and unilocular lesion attached to the cementoenamel junction of the right lower third molar. Histopathological examination of the surgically excised specimen indicated the presence of a cystic lesion lined by a reduced enamel epithelium, along with islands of hyperchromatic palisaded tumor cells with stellate reticulum-like arrangement scattered in a fibrous stroma in the deep portion of the oral mucosa. The initial diagnosis was an odontogenic hamartoma, but further analysis using immunohistochemistry for BRAF V600E and PCR to analyze the mutation in codon 600 of *BRAF* confirmed the diagnosis of incipient ameloblastoma. The patient has been under observation for 1 year and four months with no signs of recurrence.

Keywords: Odontogenic; Ameloblastoma; Mandible; Third molar.

INTRODUCTION

Hamartomas are benign tumor-like proliferations that contain an unusual combination of cells and tissues typically found in the site of occurrence[1-3]. Hamartomas are commonly found in organs such as the lung, pancreas, spleen, liver, and kidney, but they are uncommon in the head and neck area. Within the oral cavity, hamartomatous proliferations can arise from various endogenous tissues, including odontogenic and non-odontogenic epithelial derivatives, smooth and skeletal muscle, bone, blood vessels, nerves, and fat[1,4].

Odontogenic hamartomas (OH) are derived from residual epithelium of the tooth-forming apparatus including remnants of dental lamina in the alveolar bone, rests of Malassez in the periodontal ligament (Hertwig's root sheath remnants), rests of enamel organ within the jawbone, and rests of Serres in the gingiva, and the same source that gives rise to odontogenic cysts and tumors[5]. Because of their shared embryological origin, OH can microscopically resemble odontogenic cysts and tumors and may represent the initial developmental phase known as "incipient" lesions[6,7]. The term "incipient" ameloblastoma is used to describe the early stages of ameloblastoma, considering its origin. Vickers and Gorlin proposed the criteria to improve the diagnosis of incipient ameloblastoma, as there are significant similarities with odontogenic hamartomatous proliferation[8].

Statement of clinical relevance
Clinicians and pathologists must accurately identify incipient ameloblastomas to ensure appropriate treatment. Detecting the BRAF mutation is key to confirming the diagnosis and enabling timely, effective interventions.

Developing strategies to differentiate between OH and odontogenic neoplasms with aggressive behavior in the initial stage is a clinically relevant and important

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challenge for oral pathologists[6,7,9]. Previous literature has reported only a few cases concerning the diagnosis of incipient ameloblastoma based on prior analysis of OH. In this report, we present a rare case of an incipient ameloblastoma that initially resembled an OH and demonstrated the presence of the *BRAF* mutation. Additionally, we discuss the importance of conducting an expanded study to evaluate the occurrence of odontogenic cysts and tumors in their early stages when OHs are observed.

CASE REPORT

An 18-year-old man presented with a 3-week history of pain and suppuration in the right posterior mandible. The patient denied having any systemic comorbidities, and no apparent abnormalities were found on general physical evaluation. Intraoral examination revealed erythema, edema, purulence, and tenderness to palpation in the right posterior mandible near the third molar region. A computed tomography (CT) scan showed a well-defined, unilocular, hypodense lesion attached to the cementoenamel junction of the unerupted, right, mandibular third molar. The tooth was closely associated with the mandibular canal (Figures 1A-C). Based on the CT scan, the clinician suggested a provisional diagnosis of an infected dentigerous cyst.

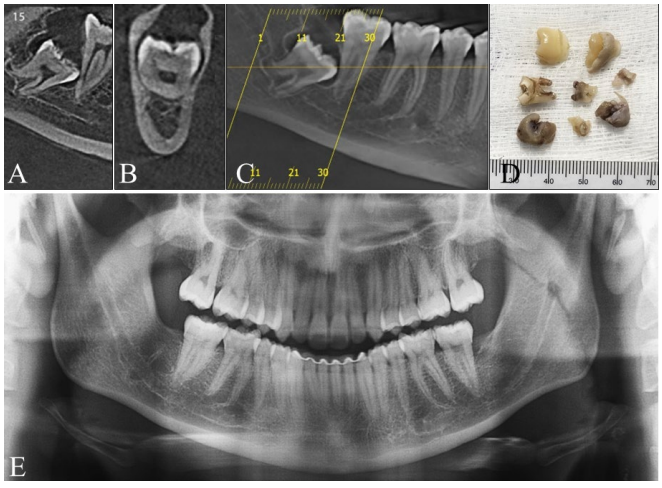

Figure 1. Computed tomography (CT) scan, gross macroscopy and follow-up. A) CT scan showed a hypodense, well-defined, and unilocular lesion that appeared to be connected to cementoenamel junction of the unerupted right mandibular third molar. B) Coronal CT scan evidencing association of the tooth roots with mandibular canal. C) Sagittal CT scan showed a hypodense and well-delimitated lesion, consistent with a dentigerous cyst. D) Upon gross macroscopic examination, a tan thickening was detected in the wall of the cystic lesion that was associated with the cementoenamel junction of the right mandibular third molar. Additionally, several fragments of soft tissue and teeth resulting from the extraction were also evidenced. E) Panoramic radiograph after 1 year and four months of follow-up with no signs of recurrence.

The surgical procedure involved enucleation of the cystic lesion and extraction of the third molar. During the procedure, a tan-colored thickening was observed in the wall of the cystic lesion associated with the cementoenamel junction. Multiple fragments of soft tissue and teeth resulting from the extraction were also observed (Figure 1D). Histopathological examination revealed fragments of a cystic capsule that was lined by a bilayered epithelium composed of eosinophilic columnar cells resembling ameloblasts and cuboidal basal cells, suggestive of the reduced enamel epithelium. In the deep portion of the oral mucosa, there were scattered islands of odontogenic epithelial cells in a fibromyxoid stroma. Some of these islands were predominantly composed of polyhedral cells, while others had peripheral palisaded hyperchromatic columnar cells and central polyhedral cells. The outer cells showed a more basophilic staining compared to the inner cells. The aggregate of odontogenic epithelial cells was confined to the connective tissue and did not infiltrate into the bone (Figure 2A-F). The initial diagnosis of odontogenic hamartoma was made. However, due to the microscopic similarity of the lesion with ameloblastoma, further investigation was conducted with immunohistochemistry and molecular analysis.

Immunohistochemical analysis for BRAF V600E was performed on 3-µm paraffin-embedded sections using established protocols. Tris/EDTA buffer solution (pH 9) (Carpinteria, Dako, CA, USA) was used for antigen retrieval by heating in a microwave to the boiling

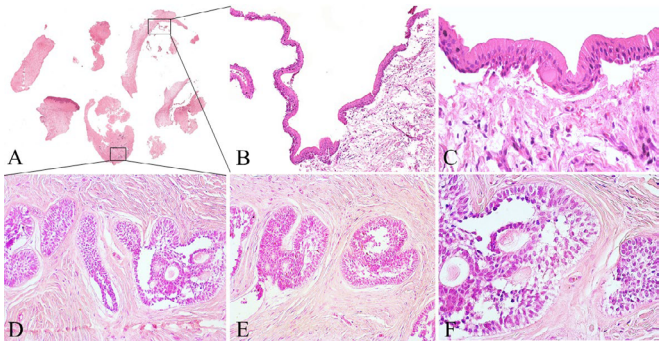

Figure 2. Microscopic analysis. A) Histological sections showing multiple fragments of soft tissue with areas of surface epithelium and cystic capsule (H&E, 50x). B) Fragment of cystic lesion associated with fibrous stroma (H&E, 200x). C) The surface epithelium in the cystic region showed columnar and cuboidal basal cells with prominent eosinophilic cytoplasm (H&E, 400x). D) Fragment of the lesion showing islands of odontogenic epithelial cells scattered in a fibrous stroma (H&E, 100x). E) Islands of odontogenic epithelium evidencing palisaded hyperchromatic columnar cells and central polyhedral cells (H&E, 200). F) The islands of epithelium cells were mainly formed by polyhedral cells with peripheral rows of palisaded columnar cells displaying reverse nuclear polarity and subnuclear vacuolization (H&E, 400x).

point for 15 minutes. The slides were allowed to cool at room temperature for 15 minutes before incubating with the Rabbit monoclonal BRAF V600E mutation-specific antibody VE1 (Recombinant RabMAb, Anti-B Raf antibody [EP152Y], ABCAM, Cambridge, Code# ab33899) at a dilution ratio of 1:150 for 1 hour and 30 minutes at room temperature. Positive controls consisted of malignant melanoma with a confirmed BRAF V600E mutation, while the negative control involved omitting the primary antibody and using phosphate-buffered saline. The results indicated that the analyzed sample was positive for BRAF V600E-specific monoclonal antibody (Figure 3A-B).

PCR was used to amplify the complete region of the *BRAF* gene, including codons 600 and 464-469. The reaction mixture contained PyroMark PCR Master Mix (12.5 µl), CoralLoad Concentrate (2.5 µl), PCR Primer (1 µl), and Water (4 µl). The initial denaturation was performed at 95°C for 15 minutes, followed by 40 cycles of 95°C for 20 seconds, 53°C for 30 seconds, and extension at 72°C for 20 seconds. Finally, there was a final extension at 72°C for 5 minutes. Unmethylated control DNA was included as a positive control, while a negative control was used in the absence of template DNA. The amplified products were immobilized, washed, and denatured before being subjected to pyrosequencing using the PyroMark Q24 system (Qiagen, PyroMark Q24 MDx V2.0, Germany). The PyroMark-Q24 software (Qiagen, PyroMark Q24 MDx (version 2.0), Germany) was used to identify the presence and percentage of specific mutations. The manufacturer-supplied limits of detection (LOD) thresholds were used to call a mutation for LOD studies (≥ % LOD is positive). Real-time curves and programs were analyzed according to the kit instructions, and the PyroMark ID software (Qiagen) was used to determine the mutant allelic frequency based on the relative peak height. The results showed that the analyzed sample presented mutations in codon 600 in *BRAF* (Figure 3C).

Collectively, these findings were consistent with the diagnosis of incipient ameloblastoma. The patient is still under follow-up; no signs of recurrence were observed after 1 year and four months (Figure 1E).

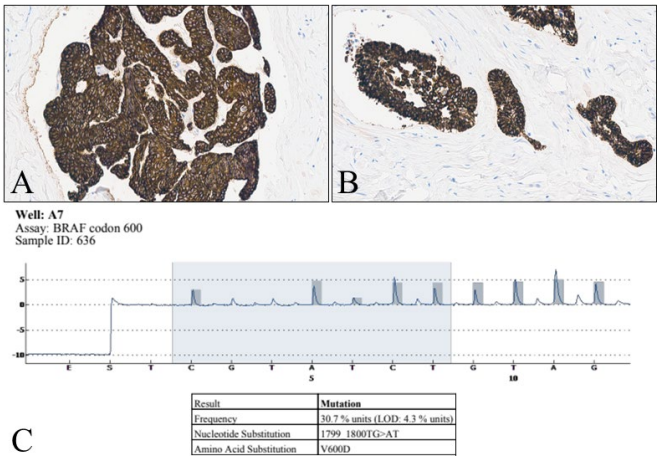

Figure 3. Immunohistochemistry and molecular analysis. A-B) Positive immunohistochemistry reaction for BRAF p.V600E-specific monoclonal antibody VE1 (200x). C) Result of the detection of *BRAF* mutation.

DISCUSSION

The diagnosis of early-stage odontogenic lesions continues to pose a challenge for oral pathologists. The rarity and challenge of diagnosing aggressive odontogenic tumors in their initial stages is notable and warrants further consideration, especially when lesions are similar to odontogenic hamartomatous proliferation[1-3]. In the present report, we present a rare case of an incipient ameloblastoma which was initially diagnosed as an odontogenic hamartoma in an 18-year-old male patient. Further analysis using immunohistochemistry for BRAF V600E and PCR analysis of mutation in codon 600 of *BRAF* led to the final diagnosis.

The development of teeth involves coordinated interactions between epithelial and mesenchymal tissues. However, when these interactions are disrupted, as is seen in odontogenic tumors, the resulting histopathological features can vary greatly. The origin of ameloblastoma can be traced back to either the enamel organ, remnants of the dental lamina, or the epithelium of a developmental odontogenic cyst[2,10,11]. Meanwhile, due to its embryological origin, ameloblastoma in its early stages may be misdiagnosed as odontogenic hamartomas, since the lesions generally do not present all the typical features of ameloblastoma[2,10].

According to the World Health Organization, ameloblastoma consists of islands of cells with peripheral columnar cells surrounding central cells that resemble the stellate reticulum. The peripheral cells are hyperchromatic, arranged in a palisade pattern, and have nuclei that are displaced from the basement membrane. Additionally, their cytoplasm is vacuolated, while the central cells are loosely arranged and may form cysts[12,13]. However, in some cases, including incipient lesions, these typical features may not be present, or they may fall within the spectrum of both odontogenic hamartomatous proliferation and neoplastic diseases, as observed in the present report[14].

Identifying that more specific molecular studies were necessary due to the hamartomatous appearance of the lesion posed the greatest challenge in diagnosing the present case. The BRAF p.V600E mutation has been observed in a significant percentage of conventional (64%), unicystic (81%), and extraosseous (63%) ameloblastomas[15]. Although BRAF p.V600E-specific monoclonal antibody VE1 is used as an alternative method for detecting BRAF p.V600E mutation through immunohistochemistry (IHC), its results must be interpreted cautiously due to the possibility of false positives[15-17]. To avoid ambiguous interpretation, the current case observed positive staining in the immunohistochemistry reaction for BRAF p.V600E and also detected *BRAF* mutation. Additionally, mutations in other components of the MAPK pathway, such as *KRAS*, *NRAS*, *HRAS*, *FGFR2*, and *EGFR*, have been found in ameloblastomas. Furthermore, non-MAPK pathway mutations, including *SMO*, *SMARCB1*, *PIK3CA*, among others, have been detected in a small number of lesions, often co-occurring with MAPK pathway mutations[16,17].

Incipient ameloblastoma is a rare but significant diagnosis, particularly for those that resemble other odontogenic lesions under clinical evaluation. This type of lesion usually does not invade bone significantly, and no report of recurrence has been previously published[2,18-20]. Although insufficient evidence supports any specific management techniques for incipient ameloblastoma, surgical excision is generally recommended[18]. Additionally, long-term follow-up is necessary to detect any recurrence. Further research is needed to gain a better understanding of the pathophysiology and epidemiology of this condition[2,18-20].

In addition, it is important to address the terminology surrounding odontogenic hamartomas, as these benign lesions can sometimes be confused with true neoplasms like ameloblastomas[4,6,7]. Odontogenic hamartomas are developmental anomalies characterized by disorganized proliferation of odontogenic tissues such as enamel and dentin, with limited growth potential and no malignant progression[6,7]. In contrast, ameloblastomas are true neoplasms with invasive potential and a risk of recurrence[8,11]. The term "hamartoma" has historically been applied to certain odontogenic lesions like odontomas due to their developmental nature, but these differ significantly from ameloblastomas in both clinical and histopathological features[6,7]. Clarifying these terms help avoid diagnostic confusion and ensures appropriate management, as ameloblastomas typically require surgical intervention, while hamartomas are often managed conservatively[6,7,20].

CONCLUSION

In conclusion, the present study presents the importance of considering ameloblastoma in its initial phase in cases of odontogenic hamartomatous proliferation. Because the microscopic aspects are not always distinguished, the lesion may represent an ameloblastoma in its initial phase, requiring confirmation of the presence of the *BRAF* gene mutation.

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AUTHORS' CONTRIBUTIONS

LLS: data curation, formal analysis, investigation, methodology, visualization, writing – original draft, writing – review & editing. ALOCR: data curation, formal analysis, investigation, methodology, visualization, writing – original draft, writing – review & editing. ALAN: data curation, formal analysis, methodology, visualization, writing – review & editing. CDS: formal analysis, methodology, visualization, writing – review & editing. JMW: conceptualization, formal analysis, methodology, validation, visualization, writing – review & editing. PAV: conceptualization, formal analysis, funding acquisition, methodology, project administration, resources, supervision, validation, visualization, writing – review & editing.

CONFLICT OF INTEREST STATEMENT

Funding: This study was financed by São Paulo State Research Foundation to support the present study (FAPESP #22/03123-5).

Competing interests: The authors have no relevant financial or non-financial interests to disclose.

Ethics approval: As this study is based on a retrospective case report, approval from the ethics committee was not required, in accordance with institutional guidelines.

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