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| ORIGINAL

Epithelial-mesenchymal markers and their correlation with clinical aspects in odontogenic keratocysts

Marcadores epitélio-mesênquima e sua correlação com aspectos clínicos em lesões de ceratocisto odontogênico

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ABSTRACT

Objective: Odontogenic keratocysts have a high recurrence rate and aggressive clinical behavior. The event called epithelial-mesenchymal transition is a process in which the epithelial cell loses its epithelial characteristics and acquires properties typical of mesenchymal cells. Studies have already demonstrated that odontogenic keratocysts has expression of tumor markers, but the lack of clarification about its development mechanism and molecular composition makes the therapeutic options remain limited. The aim of this study is to evaluate the expression of epithelial-mesenchymal transition marker proteins in these lesions, correlating the expression of these proteins with clinical aspects of each case. **Methods**: Patients with odontogenic keratocysts diagnoses, treated by the Department of Oral and Maxillofacial Surgery of the Erasto Gaertner Hospital, Curitiba, Brazil in the period between 2016 and 2019 were evaluated by immunohistochemical analysis, to assess the expression of epithelial-mesenchymal transition markers (Vimentin, beta-catenin and E-cadherin) by qualitative analysis. **Results**: Eighteen patients were included, with a mean age of 43 years, and most of them were male. The mandible was more affected than the maxilla. No association between the clinical characteristics of the cysts and the immunohistochemical profile for epithelial-mesenchymal transition proteins was observed. **Conclusion**: The positivity of E-cadherin and negativity of vimentin demonstrates that its function is preserved. Loss of function of E-cadherin is associated with worse prognosis. The identification of the epithelial-mesenchymal transition process as a prognostic marker for odontogenic cysts and tumors could be an important tool for defining treatment.

Indexing terms: Epithelial-mesenchymal transition. Odontogenic cysts. Odontogenic keratocysts.

RESUMO

***Objetivo**: O ceratocisto odontogênico têm uma alta taxa de recorrência e comportamento clínico agressivo. O evento chamado transição epitelial-mesênquima (TEM) é um processo no qual a célula epitelial perde suas características epiteliais e adquire propriedades*

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típicas das células mesenquimais. Estudos já demonstraram que o ceratocisto odontogênico tem expressão de marcadores tumorais, mas a falta de esclarecimento sobre seu mecanismo de desenvolvimento e composição molecular faz com que as opções terapêuticas permaneçam limitadas. O objetivo deste estudo é avaliar a expressão das proteínas marcadoras de transição epitelial-mesênquima nestas lesões, correlacionando a expressão destas proteínas com os aspectos clínicos de cada caso. ***Métodos****: Os pacientes com diagnóstico de ceratocisto odontogênico, tratados pelo Serviço de Cirurgia Bucomaxilofacial do Hospital Erasto Gaertner, Curitiba, Brasil, no período entre 2016 e 2019, foram avaliados por análise imunohistoquímica, para avaliar a expressão dos marcadores transição epitelial-mesênquima (Vimentina, beta-catenina e E-cadherina).* ***Resultados****: Foram incluídos 18 pacientes, com idade média de 43 anos, e a maioria deles eram do sexo masculino. A mandíbula foi mais afetada do que a maxila. Não foi observada associação entre as características clínicas dos cistos e o perfil imuno-histoquímico das proteínas transição epitelial-mesênquima.* ***Conclusão****: A positividade da E-caderina e a negatividade da vimentina demonstram que a sua função está preservada. A perda da função da E-caderina está associada a um pior prognóstico. Identificar o processo da transição epitelial-mesênquima como um marcador de prognóstico para cistos e tumores odontogênicos pode ser uma ferramenta importante para definir o tratamento dessas lesões.*

Termos de indexação: *Transição epitélio-mesênquima. Cistos odontogênicos. Ceratocistos odontogênicos.*

INTRODUCTION

The term "odontogenic" refers to lesions derived from elements of the epithelium, ectomesenchyme and/or mesenchyme that participated or are participating in the formation of the dental apparatus. These lesions are found exclusively in the maxillofacial region and can occur at any age [1].

Odontogenic keratocysts (OK) present a distinct form of development from other odontogenic cysts. According to the literature, the mandible is involved in 60-80% of OK cases, with a greater tendency to involve the posterior body and ascending ramus [2], affecting mainly male patients in the second, third and fourth decades of life [3]. Most cases are sporadic lesions, occurring singly mainly in the mandible, with a high recurrence rate and aggressive clinical behavior [2,4,5].

The etiology of OK is probably associated with the development of the dental lamina and its remnants, however, despite being intensively studied, the pathogenesis of this lesion still holds unanswered questions [2]. Due to its peculiar characteristics, this lesion has long been a matter of debate, being reclassified numerous times as cyst or tumor [6,7].

The event called epithelial-mesenchymal transition (EMT) is a complex process in which the epithelial cell loses its epithelial characteristics and acquires properties typical of mesenchymal cells. In this way, the cell increases its ability to invade, migrate, and generate metastasis. The dissociation of tumor cells across tissues due to changes in cell-cell adhesion is one of the main causes of the tumor's invasive capacity [8], being a possible explanation for the aggressive behavior of OK.

The concept of EMT in tumor metastasis formation is based on observations that epithelial carcinoma cells that acquired mesenchymal markers such as vimentin and loss of cell-cell adhesion molecules such as E-cadherin [9] were associated with a higher metastatic potential. Currently, the most commonly used markers for EMT verification are vimentin, fibronectin and N-cadherin (mesenchymal markers), E-cadherin (epithelial markers) and Snail and Slug (transcription factors) [8,9].

Evidence for the involvement of EMT in OK progression is still limited [8], however, dysregulation of these proteins may explain the molecular mechanism by which these lesions develop. E-cadherin is the protein member which connects epithelial cells together at adherens junctions and it plays an important role in epithelial cell adhesion and the loss of its function is a major contributor to cancer progression because the majority of solid tumors are carcinomas that arise from epithelial tissue [10]. On the other hand, Vimentin expressions increased as the tumor progressed from well to poorly differentiated [11]. Detection of this protein's expression is a promising application for clinical diagnosis, prognosis and therapeutics in cancer and aggressive benign lesions.

The aim of this paper is to survey the cases of OK diagnosed at the Department of Oral and Maxillofacial Surgery of the Erasto Gaertner Hospital (EGH) in the period between 2016 and 2019 and evaluate the expression of EMT marker

proteins in these lesions, correlating the expression of these proteins with clinical aspects of the lesions and with the prognosis of each case.

METHODS

The study was approved by the Research Ethics Committee of EGH under protocol CAE 24601119.0.0000.0098 and was conducted respecting resolution 466/12 CONEP. The cases of OK diagnosed and treated at the Department of Oral and Maxillofacial Surgery of the EGH, Curitiba, Brazil, during the years 2016 to 2019 were surveyed. The cases were surveyed through electronic medical-hospital records, from which clinical information such as gender, age, race, year of diagnosis, location and size of the lesion, type of treatment used, use of Carnoy's solution, and occurrence or not of recurrence were retrieved.

All patients who underwent surgical treatment at EGH from 2016 to 2019 were included. Syndromic patients, cases in which the data in the medical records were incomplete, or who underwent treatment outside EGH were excluded.

The histopathological slides of each case, stained in Hematoxylin and Eosin, were selected and analyzed again to confirm the diagnosis of OK. The paraffin blocks from each slide were then selected and immunohistochemical analysis was requested to evaluate the expression of EMT markers, which were the proteins Vimentin, beta-catenin and E-cadherin for each of the cases.

Immunohistochemical reaction was performed on 3-µm thick sections. Antigen recovery was achieved with Tris/EDTA buffer solution (pH 9) (DakoCytomation®, Carpinteria, USA). Primary antibodies used were vimentin, beta-catenin and E-cadherin (DakoCytomation®, Carpinteria, USA) which were incubated for 90 min in a humid chamber at 25 °C. Envision Dual Link System kit (HRP®, DakoCytomation®, Carpinteria, USA) was used with diaminobenzidine as chromogen agent. Negative control was included in all batches.

Clinical findings and data resulting from immunohistochemical analysis were described in a table to identify possible associations between clinical behavior and immunohistochemical profile of EMT proteins.

RESULTS

The study included 18 patients with a mean age of 43 years, most of them male (61.11%) and all of them were Caucasian. The mandible was more affected when compared to the maxilla (72.22%) and the lesions had an average of 3 cm in their largest diameter.

Only one patient presented lesion recurrence in the mandible. The other patients did not present lesion recurrence during the period analyzed in this study and are still under follow-up. As for treatment, cystic enucleation by curettage was the surgical treatment performed in all cases, and of these, 8 cases (44.44%) was associated with transoperative Carnoy's solution application.

In the immunohistochemical analysis, positivity of the markers E-cadherin (figure 1) and beta-catenin (figure 2) was observed for all cases, in the epithelium, both maxilla and mandible. Regarding the Vimentin protein, all cases were negative (figure 3) and the expression was only seen in the connective tissue. For all three markers, no abnormal expression was observed, suggesting normal function of the proteins. No association between the clinical features of the cysts and the immunohistochemical profile for EMT proteins was observed.

DISCUSSION

Due to its peculiar characteristics and behavior, the etiopathology of OK is still in debate. In 2005, WHO classification reclassified OK as a tumor, and it was then called Keratocystic Odontogenic Tumor and defined as a "benign

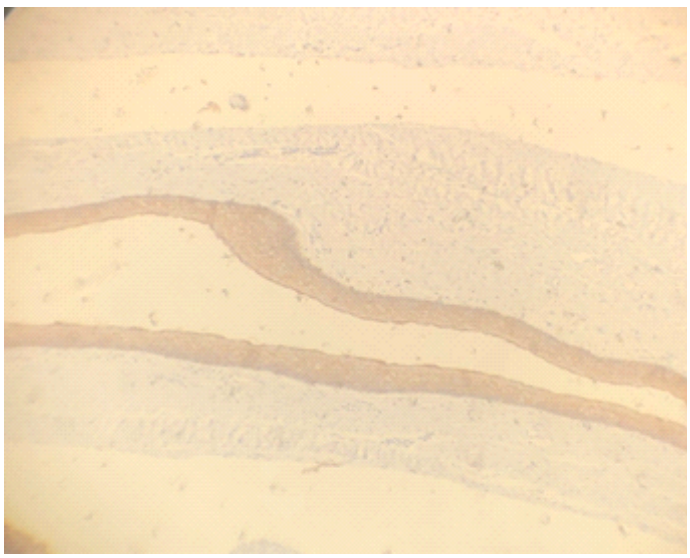

Figure 1. Immunohistochemistry aspect showing positivity of e-cadherin.

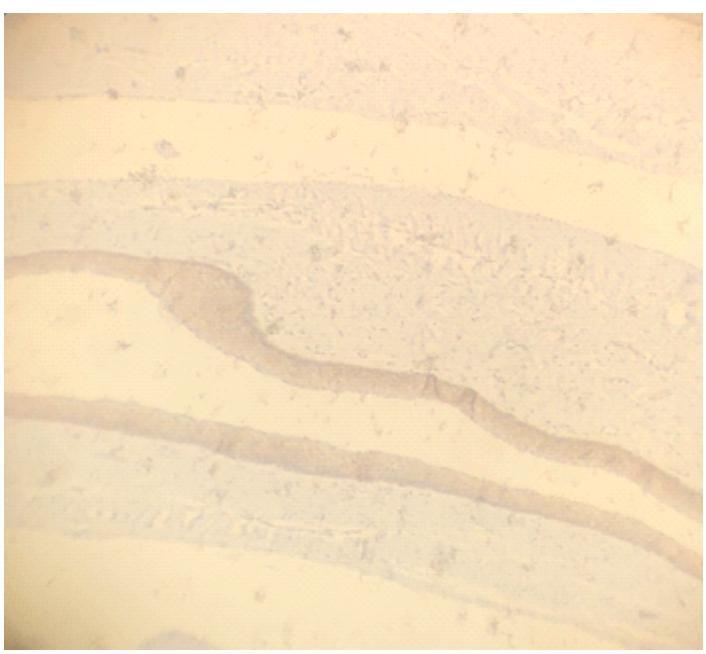

Figure 2. Immunohistochemistry aspect showing positivity of beta-catenin.

intraosseous tumor of odontogenic origin with a characteristic layer of parakeratinized stratified squamous epithelium and with a potential for aggressive and infiltrative behavior" [6]. However, the latest edition of the WHO blue book (2017), classified the OK again as an odontogenic cyst due to a debate regarding the neoplastic origin of this lesion [7]. New findings regarding its etiology and molecular profile will allow a better understanding of how this lesion develops and may mean advances in treatment and a better prognosis for affected patients.

Similar results to those reported in the literature were found in this study regarding the clinical characteristics of OK. According to Kshirsagar et al. [2] the mandible is involved in 60-80% of OK cases and affects mainly male patients in the second, third and fourth decades of life [3].

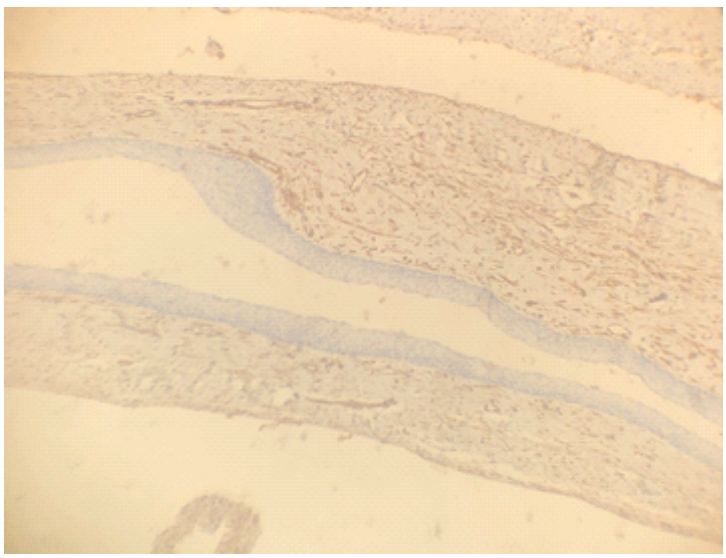

Figure 3. Immunohistochemistry aspect showing negativity of vimentin.

In relation to signs and symptoms, most patients in our study presented signs such as increased volume in the site of the lesion (55%) accompanied or not by pain symptoms (10%), while others had their diagnosis from radiographic findings and did not present any visible changes through physical examination (40% of cases). These findings corroborate with Kshirsagar et al. [2], which reported that patients may present symptoms such as pain, edema, and rarely have paresthesia in the lower lip region. On the other hand, some patients are asymptomatic until the lesion reaches large dimensions or develops pathological fractures. In many cases the lesion is only noticed through routine radiographic examinations.

Concerning treatment, options range from more conservative procedures such as marsupialization and enucleation of the lesion with or without peripheral osteotomy to more radical therapies such as segmental resection [12]. The location of the lesion (mandible or maxilla), the size, and evidence of cortical bone perforation must be considered for treatment choice [13]. In our study, all cases were treated with surgical enucleation. More radical techniques such as segmental resection is indicated in the treatment of lesions that involve the adjacent soft tissue and in which there is evidence of cortical bone disruption [14].

Conservative surgical treatment options such as enucleation and marsupialization are often associated with high recurrence rates. For this reason, the combination of these procedures with additional therapies such as cryotherapy and the use of Carnoy's Solution are described in the literature [14]. Only one of the cases reported int his study did not receive application of Carnoy's solution as an adjuvant therapy and was also the only case that presented recurrence of the lesion.

Previous studies have shown that EMT is associated with aggressive behavior of OKs [15]. Although no association was observed between the variables analyzed and the immunohistochemical profile of EMT, the positivity of E-cadherin protein and negativity of vimentin demonstrates that its function is preserved, as well as beta-catenin. Both proteins have cell adhesion function and they are part of the Wnt signaling pathway, with tumor suppressor function. Loss of function of E-cadherin is associated with worse prognosis and survival in different cancers, mainly associated with tumor invasion, apoptosis, cell cycle, and differentiation [10]. The identification of the EMT process as a prognostic marker for odontogenic cysts and tumors could be an important tool for defining treatment and follow-up of these patients.

The positive expression of beta-catenin and e-cadherin observed in this study suggest a preserved cell-adhesion function, although it was a small sample. Different biomarkers were studied in the intent to understand the biological behavior of this lesion and to guide clinical decision. While the best biomarker has not yet been identified, clinical management must always include some adjuvant treatment, in the case of this study, Carnoy's solution, since the aggressiveness and potential of recurrence of this lesion is its most challenging characteristic.

CONCLUSION

Even though it is a benign lesion, OK is associated with complications and extensive defects, especially in larger lesions, with the possibility of losing large segments of bone tissue, as well as repeated recurrences. For this reason, the study of markers that allow a better understanding of its biological behavior is important for the best management of the patient.

Collaborators

JL Vendruscolo and M Souza Lessa, conceptualization, methodology, data curation, writing. S Ossamu Ioshii, review and editing, visualization, formal analysis, supervision, project administration. JL Schussel, review and editing, formal analysis, supervision, project administration. LM Sassi, review and editing, visualization, formal analysis, supervision, project administration.

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