

Histopathological evaluation of dental follicles associated with impacted third molars

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Aim: To histologically evaluate dental follicles of impacted third molars with no radiographic evidence of pathology.

Methods: We carried out both a quantitative and qualitative analysis of pericoronal follicles removed from impacted third molars and investigated the association with clinical data. The sample included 36 extracted dental follicles of impacted third molars, obtained from 28 patients, which presented with no radiographic evidence of pathologies. **Results:** None of the follicles analyzed showed any pathological entity. The epithelial lining was observed in 61.1% of samples, being identified as reduced enamel epithelium. We found a significant relation between the the presence of inflammatory infiltrate and the group aged over 21 years (64.3%; $p < 0.05$). **Conclusions:** Considering the absence of pathological lesions, we suggest that the removal of impacted third molars, particularly in young-aged individuals, should be carefully indicated.

Keywords: impacted tooth, third molar, dental follicle, pathology

Introduction

The therapeutic removal of third molars is a common maxillofacial surgery that takes into account clinical diagnostic criteria, including the presence of symptoms or pathologies, preventive measures or orthodontic indications in asymptomatic patients^{1,2}. Several issues have been linked to the maintenance of these teeth, such as root resorption or carious lesions on the second molar, trismus, pericoronaritis, localized pain and development of cysts or tumors. In the presence of such complications, the surgical removal has been widely indicated; however, this decision becomes more difficult when there is no symptom or associated pathology^{3,4}.

Even though prophylactic surgery has been justified on the basis that third molars might have a greater potential to develop associated diseases, this practice remains largely controversial^{5,6}. Some authors have argued against surgery in asymptomatic impacted teeth based on the low incidence of pathologies related to third molars. Most studies have used data obtained only through radiographic analysis; nevertheless, the prevalence of pathological conditions may be higher than usually is expected on radiographies^{7,8}. Thus, only radiographic aspects are inconclusive for the proper diagnosis of lesions associated with impacted third molars⁹.

Moreover, although histological changes may exist in the surrounding tissues of impacted teeth, there is no accurate information regarding the prevalence of diseases in the pericoronal tissue, which is thought to range from 10% to 58.5%^{2,10}. Hence, further research is necessary to identify a prevalence of alterations that can justify the prophylactic removal of impacted teeth. In this way, we aimed to histologically evaluate dental follicles of impacted third molars with no radiographic evidence of associated pathologies.

Material and Methods

The present study was approved by the Research Ethics Committee of the Federal University of Maranhão (protocol #23115.016756/2011-40). We conducted clinical, morphological and radiographic evaluations of dental follicles of impacted third molars with no radiographic evidence of associated pathologies. The sample included 36 dental follicles obtained from 28 patients seeking orthodontic treatment in a private clinic in São Luís, Brazil.

Inclusion criteria were the presence of impacted third molars with an indication for extraction and a dental follicle radiographic space of up to 2.5mm¹⁰. Patients with any local or systemic inflammatory and/or infectious disease and those who did not agree to participate in the study were not included. Data obtained from each patient included age, gender, dental unit extracted, and indication for surgery.

The radiographic evaluation was carried out by a specialist in a blind fashion, using scanned panoramic radiographies at the ratio of 1:1 that were processed by the Radiocef Studio 2 software (Radio Memory, Belo Horizonte, MG, Brazil). The width

of the pericoronal space was used as the largest distance from the crown to the surrounding bone. The Winter's classification of impaction was also registered.

For morphological analysis, dental follicles obtained during the extraction of third molars were fixed in 10% formaldehyde solution and embedded in paraffin. Histological cuts (5 μ m) were performed, stained using the hematoxylin-eosin technique and observed on a microscope. The histopathological analysis of dental follicles was carried out by two independent oral pathologists that were blind to clinical and radiographic data. The Cohen's kappa coefficient (κ) was calculated using SPSS 18.0 (IBM Corp, Armonk, NY, USA).

The following characteristics were taken into account for the analysis of dental follicle specimens. Connective tissue: dense, loose, or myxoid, which may present occasional mineralization; epithelial odontogenic residues; and unspecific inflammatory infiltrate. Limiting epithelium: reduced enamel epithelium or absence of the epithelial lining. The presence of a stratified squamous epithelium was considered normal, with up to 3 cell layers, fragmented or separated from the surrounding connective tissue¹¹ (Figure 1).

Data were analyzed using SPSS 18.0. To verify statistically significant associations among variables, the Pearson's chi-squared test (χ^2) was utilized at a 5% significance level.

Results

A total of 36 pericoronal follicles of impacted third molars were collected from 28 patients, aged 14-37 years (mean age: 21.1 years). A total of 55.6% of follicles were obtained from females (n = 20) and 44.4% were from males (n = 16). Mean age for

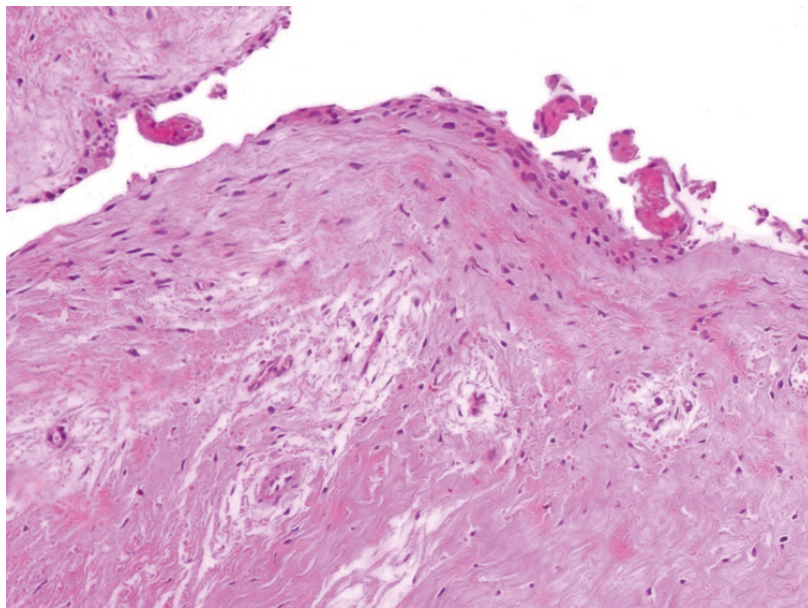


Figure 1. Histological analysis of a dental follicle from a patient under 21 years old: a) limiting enamel epithelium exhibiting discontinuity. b) connective tissue fragment. (original magnification, $\times 40$; HE stain).

females was 19.75 years (95% CI: 18.58–20.92) and 22.88 (95% CI: 19.48–26.27) for males. The sample was then divided into two age groups: under 21 years old and over 21 years old. Most teeth included in the present study were lower left third molars (38.9%), followed by lower right third molars (36.1%).

In relation to the third molars impaction type, according to the Winter's classification, 47.2% were mesioangular, 30.6% vertical, 11.1% distoangular, and 11.1% horizontal. In regard to their location, 75% of all impacted teeth were in the mandible (Table 1). We found no association between classification and location with histological features.

Regarding the histopathological analysis, inter-rater agreement was satisfactory ($\kappa = 0.84$). Microscopically, 38.9% of the dental follicles did not present epithelial lining, while 61.1% presented with a fragmented reduced enamel epithelium or separated from the surrounding connective tissue. The connective tissue exhibited altered myxomatosis in 72.2% of cases, while epithelial odontogenic residues and calcification were found in 88.9% and 36.1%, respectively, but there was no association between these variables and the age group ($p > 0.05$; Table 2).

We observed the presence of nonspecific chronic inflammation in 41.7% of samples to be significantly associated with the age range of patients. The presence of inflammatory infiltrate was positive in both groups, but was higher in patients aged over 21 years old ($p = 0.028$) as shown in Table 2.

Table 1. Distribution of groups according to the Winter's classification and anatomical location.

Winter's Classification	n	%
Mesial	17	47.2
Vertical	11	30.6
Horizontal	4	11.1
Distal	4	11.1
Location	n	%
Mandible	27	75
Maxilla	9	25

Table 2. Association between the histological characteristics of samples and the age group.

Age (years)	Myxomatous áreas		p	Epitelial odontogenic residues		p	Calcifications		p	Inflammatory Infiltrate		p
	Yes	No		Yes	No		Yes	No		Yes	No	
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)				
< 21	14 (53.8)	8 (80.0)	0.14	19 (59.4)	3 (75.0)	0.54	8 (61.5)	14 (60.9)	0.96	6 (27.3)	16 (72.7)	0.02*
> 21	12 (46.2)	2 (20.0)		13 (40.6)	1 (25.0)		5 (38.5)	9 (39.1)		9 (64.3)	5 (35.7)	

* $p < 0.05$, according to the Pearson's chi-squared test.

Discussion

From data obtained, we observed an increased frequency of surgical removal of impacted third molars in female patients aged over 21 years old, corroborating with previous findings^{10,11}. Still, there was a higher incidence in the mandible as 75% of impacted teeth were lower third molars, similarly to the findings of Stathopoulos et al.⁵ (2011). Regarding the radiographic evaluation, we considered as a normal pericoronal space a width of up to 2.5mm. According to the literature, the normal space varies from 2mm to 4mm; however, it is important to correlate the radiographic findings with clinical and histopathological aspects³.

Small cystic histological alterations may be present in dental follicles tissue and not be featured on radiographies, and the reverse may also occur if the enlargement of the pericoronal space represents a normal tissue on the histopathological exam¹⁰. Regarding the dental follicle, Stathopoulos et al.⁵ (2011) described a high transformation potential in cysts or neoplasms. On the other hand, Khorasani and Samiezhadeh¹² (2008) reported that this potential may be overrated as dental follicles can be misdiagnosed as pathological lesions, considering that the distinction between a small dentigerous cyst and a large dental follicle may be difficult, requiring thereby histological, clinical and radiographic correlation. Immunohistochemical and molecular tests may be useful tools for differential diagnosis among these follicular alterations¹³. In this study, all dental follicles analyzed presented without any pathological entity.

Histologically, the dental follicles analyzed were identified as elements of fibrous connective tissue with different amounts of altered myxomatosis, epithelial calcification, epithelial odontogenic residues and epithelial lining. The incidence of epithelial lining on dental follicles has been reported in 69-87.8% of cases^{12,13}. Here, we observed the presence of epithelium (reduced enamel epithelium) in 61.1% of samples, most probably due to the average age of patients (21.1 years). The reduced enamel epithelium is more common in young individuals, whereas the incidence of squamous epithelium is regularly found in older subjects.

A significant relation between the increasing age of patients and the presence of inflammatory infiltrate in the connective tissue was observed. Prior studies that investigated the connection between the inflammatory infiltrate and oral tissues proliferation found inflammation to cause chronic irritation, stimulate epithelial cells proliferation and modify a normal epithelium into a squamous epithelium, which is more resistant to external factors¹⁴. Metaplasia can be considered an adaptive reaction in cells vulnerable to stress, improving their tolerance to inadequate environmental conditions¹⁵, but this epithelial type was not found in the present study.

Furthermore, 88.9% of samples presented odontogenic epithelium inside the connective tissue, more than the 79% reported by Kim and Ellis¹⁶ (1993). A similar reduction of epithelial islands in relation to increasing age was also reported in this study; however, this relation was not significant. Our data shows that only 27.8% of dental follicles presented with myxomatous areas as compared to 40% reported by Khorasani and Samiezhadeh¹² (2008). A significant relationship between altered myxomatosis, age and gender was not found.

In conclusion, although our histopathological evaluation of dental follicles did not show pathological alterations, the participants aged over 21 years presented with unspecific chronic inflammation. Therefore, we suggest that impacted third molars should be

extracted only due to pathological processes that may influence the patient's well-being or by specific indications, especially in young-aged patients. We also encourage further studies that include immunohistochemical and molecular analysis.

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