

Correlation between inflammatory infiltrate and epithelial lining in 214 cases of periapical cysts

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Abstract: The aim of this study was to evaluate the prevalence of periapical cysts, identify their clinical and microscopic features and correlate their microscopic features with the inflammatory infiltrate present in the lesion site. A total of 214 cases were collected over a 10-year period. Clinical data, including gender, age, race, symptoms and location of the lesion, were recorded. Two independent examiners with no prior knowledge of the patients' clinical data conducted the microscopic evaluations. Statistical analyses were performed using Fisher's or chi-square tests at a 5% level of significance. The results showed that periapical cysts were more prevalent in white women, with a mean age of 35 years, and in the anterosuperior region. The majority of the lesions were lined by atrophic cystic epithelium, which was associated with moderate inflammatory infiltrate in the cystic capsule ($p < 0.01$), with a diffuse localization pattern ($p = 0.03$) and absence of neutrophils ($p = 0.01$). Our findings suggest that periapical cysts lined by atrophic epithelium are related to the presence of moderate mononuclear inflammatory infiltrate.

Descriptors: Inflammation; Radicular Cyst; Biopsy.

Introduction

Periapical cysts—also known as radicular, periradicular or apical periodontal cysts—are inflammatory jaw cysts on teeth with infected and necrotic pulp. The cysts are a direct sequela of apical granulomas, although not every apical granuloma will develop into a cyst.¹ Radiographically, a periapical cyst has an imaging appearance similar to that of a periapical granuloma. Although cysts are often larger than granulomas, the size of the lesion cannot be used as a definitive criterion for diagnosis. As the lesion becomes larger, it is more likely to be a cyst.² Cystic transformation occurs owing to inflammatory stimulation of the epithelial rests of Malassez in the periodontal ligament, secondary to a root canal infection, in which the bacteria and their products, that were previously confined to the dental pulp, go beyond the root canal system and reach the periapical tissues.^{1,3}

Periapical cyst formation seems to be induced by an acute inflammatory reaction,⁴ although mononuclear cells are commonly found in the inflammatory infiltrate.⁵ T- and B-lymphocytes and macrophages constitute most of the inflammatory cells involved.⁶ Macrophages are observed primarily in active inflammatory sites, mainly in the subepithelial region

and seldom in the surrounding area.⁷ Additionally, the thin and thick cystic capsules present differences with regard to the inflammatory cells involved. Thin capsules have a predominance of lymphocytes, whereas thick capsules are predominantly composed of plasma cells and macrophages.⁸ Inflammatory cell count and degree of vascularization in periapical cysts have shown a strong association with increased expression of tumor necrosis factor-alpha (TNF- α),⁸ indicating that inflammatory cells may have an important role in the development of these lesions.

The pathogenesis and growth mechanisms of inflammatory odontogenic cysts of the jaws are not fully understood.⁹ The inflammatory infiltrate in the cystic lining appears to play an important role in the pathogenesis of periapical cysts.¹⁰ Therefore, the purposes of the present study were to evaluate the prevalence of periapical cysts, identify their clinical and microscopic features and correlate their microscopic features with the inflammatory infiltrate present in the lesion.

Methodology

Records of all the cases diagnosed as periapical cysts in the 2000 to 2010 period in the Anatomic Pathology Laboratory of our institution were retrieved. Periapical cysts were defined as follows:

1. a lesion seated on the periapical region of a non-vital tooth, and
2. histologic evidence of stratified non-keratinized squamous epithelium completely or partially lining a cystic cavity or tissue fragments.

Clinical data, including gender, age, race, symptoms, duration of the lesion up to the dental consultation and location were recorded. The study was carried out after the approval of the institutional Ethics Committee (protocol #140/2010) on research in human beings.

Qualitative and quantitative microscopic analyses

The material used in this study consisted of pericystic biopsy tissues from routine cyst surgeries sent to our institution's Anatomic Pathology Laboratory

and immediately fixed in 10% neutral buffer formalin at room temperature. Five- μ m paraffin-embedded specimens were mounted on microscope slides and stained with hematoxylin and eosin (HE). Two independent examiners (J.A.C.H. and A.A.C.P.) with no prior knowledge of the patients' clinical data conducted the microscopic evaluations using an optical binocular microscope (Axiostar Plus, Carl Zeiss, Jena, Germany) equipped with a 40 \times /0.65 objective (Achromplan, Carl Zeiss, Jena, Germany), thus achieving a 400 \times magnification. Whenever a disagreement arose, a consensus approach was adopted.

The following tissue structures were evaluated:

- cystic cavity;
- cystic epithelial lining and its thickness;
- characterization, distribution and intensity of inflammatory infiltrate;
- presence of congested and hyperemic blood vessels; and
- presence of cholesterol crystals.

The quantitative microscopic analysis was performed according to the method described by Jurisic *et al.*⁸ Each specimen was graded under 400 \times magnification as:

- slight, < 10 inflammatory cells per field;
- moderate, 10–50 inflammatory cells per field, and
- intense, more than 50 inflammatory cells per field.

Grading of each specimen was based on the average inflammatory cell number in three consecutive microscopic fields starting from the epithelial-connective tissue border and proceeding gradually deeper into the lamina propria. The thickness of the epithelial lining was evaluated in respect to the number of cell layers and described as:

- atrophic, < 6 epithelial cell layers;
- normal, 6–10 epithelial cell layers; and
- hyperplastic, more than 10 epithelial cell layers.

The lesions with a variable number of layers were classified as follows:

- normal and atrophic;

- atrophic and hyperplastic; or
- normal and hyperplastic.

Statistical analysis

Statistical analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, USA) for Windows software (Microsoft, Mountain View, USA). Fisher's or chi-square tests were used for the analyses, and *p* values lower than 0.05 were considered significant.

Results

Periapical cysts corresponded to 6.21% (214 cases) of all the cases diagnosed over a 10-year period. Patient age ranged from 7 to 77 years old, with a mean age of 35 years. Regarding gender, 46.7% (110) of the periapical cysts were diagnosed in men, and 53.3% (114) in women. Additionally, most of the patients (78.7%) were white. Concerning location, the lesions occurred mainly in the anterosuperior (41.7%) and posterosuperior (31%) regions. The majority of the lesions (56.8%) had a maximum diameter of 1 cm, and 71% of them were asymptomatic. Recurrence was reported in 6.54% of all cases.

Microscopic analysis revealed that 83.6% (179) of the lesions presented partial epithelial lining, and 66.4% (142) presented atrophic lining. Atrophic epithelial lining was associated with moderate inflammatory infiltrate in the cystic capsule (Table 1), with diffuse location (*p* = 0.03) and with the absence of neutrophils (*p* = 0.01). The inflammatory infiltrate was found to be moderate in 54.7% (117) of the cases, it had a diffuse location in 65.1% (136) and was predominantly composed by lymphocytes, plasma cells and macrophages (Figure 1). Lack of neutro-

phils associated with slight inflammatory infiltrate was observed in the cystic capsule (Table 2), and, when present, they were associated with congested and hyperemic blood vessels (*p* < 0.01). Furthermore, cholesterol crystals were seen in 13.6% (29) of the lesions (Figure 2).

Discussion

The clinical diagnosis of oral lesions can be tricky owing to the similar appearance of many conditions presenting different etiology. Therefore, there is a risk of misdiagnosis and delayed treatment if the therapeutic choice is based solely on clinical aspects.¹¹ A study in the New Zealand population revealed that a correct clinical provisional diagnosis of periapical cyst was given in only 36% of the

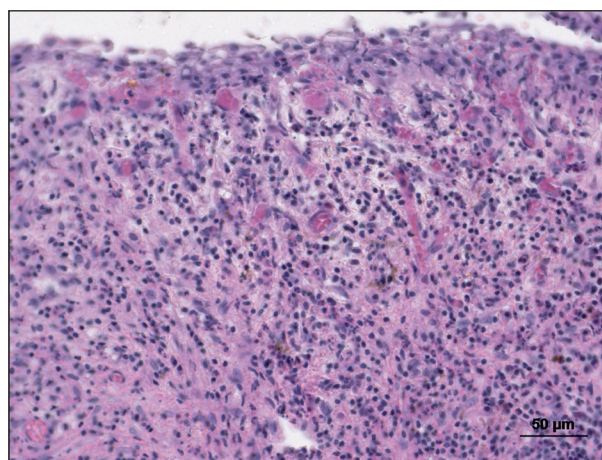


Figure 1 - Periapical cyst. Cystic wall covered by atrophic stratified squamous epithelial lining exhibiting moderate mononuclear inflammatory infiltrate (H&E; original magnification, 400x).

Table 1 - Intensity of inflammatory infiltrate according to thickness of epithelial lining in periapical cysts.

Inflammatory infiltrate	Thickness of epithelial lining					
	Normal	Atrophic	Hyperplastic	Normal and atrophic	Normal and hyperplastic	Atrophic and hyperplastic
Absent	1	0	0	0	0	0
Slight	7	36	2	6	1	0
Moderate	7	80	4	20	4	2
Intense	1	26	5	5	1	6
<i>p</i> value*	0.001					

*Chi-square test at 5% significance level.

Table 2 - Intensity of inflammatory infiltrate according to presence of neutrophils in periapical cysts.

Inflammatory infiltrate	Neutrophils	
	Absent	Present
Slight	49	3
Moderate	70	47
Intense	15	28
<i>p</i> value*	< 0.001	

*Chi-square test at 5% significance level.

cases,¹² which highlights the importance of knowing the clinical, radiographic and microscopic aspects of the lesion. Diagnostic confirmation can only be ascertained after anatomic pathology examination.¹³

According to Beconsall-Ryan *et al.*,¹² inflammatory lesions corresponded to 72.8% of radiolucent lesions of the jaws. Among them, 29.2% were periapical cysts. Among Brazilians, periapical cysts were the most frequent odontogenic cysts in adults (66.5%).¹⁴ In the present study, periapical cysts were more prevalent in white women, with a mean age of 35 years, and in the anterosuperior region, corroborating data found in the related literature.^{5,14} However, other authors have reported a similar prevalence in both genders¹² and a mean age of 44.3 years.¹⁵ These discrepancies may be attributed to differences in sample selection and size, as well as the diagnostic criteria adopted by the authors of different studies.

There is continuing controversy regarding the kind of inflammatory infiltrate present in periapical cysts. Marçal *et al.*⁵ found that mononuclear infiltrate was significantly more frequent than mixed infiltrate, and that the latter was present in lesions with fistulae. Lin *et al.*,¹⁵ however, found a mixed infiltrate in 52.1% of the lesions. Microscopic analysis of our samples revealed that the presence of congested and hyperemic blood vessels was associated with intense inflammatory infiltrate in the cystic capsule ($p < 0.01$) and with the presence of neutrophils ($p < 0.01$), features which characterize lesions in the acute inflammatory phase. However, most of the lesions presented moderate inflammatory infiltrate, which was associated with the presence of

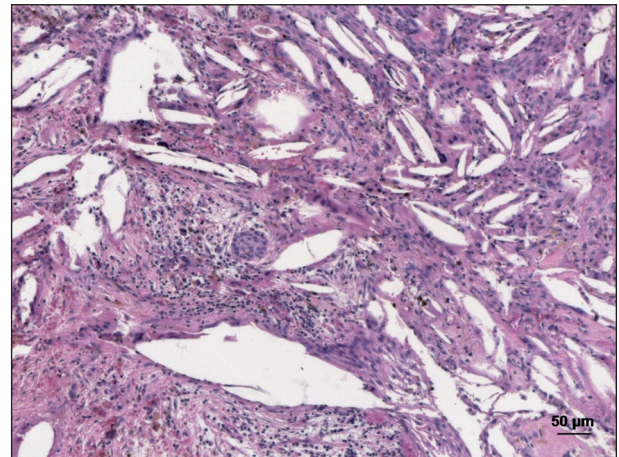


Figure 2 - Periapical cyst. Cholesterol crystals and foreign body-type reaction with giant cells present in the capsule (H&E; original magnification, 200×).

plasma cells ($p = 0.01$) and macrophages ($p < 0.01$). These results are in agreement with those of Lin *et al.*,¹⁵ and may justify the fact that 71% of the lesions in our study were asymptomatic, which is compatible with chronic inflammation (mononuclear infiltrate). The presence of plasma cells in cysts suggests a local humoral immune reaction, and indicates that the majority of the lesions were in a developing stage.⁶

Another histopathological feature evaluated was the presence of cholesterol crystals, which were observed in 13.6% of periapical cysts, a rate similar to that found by Santos *et al.*¹⁶ The major source of cholesterol may be from locally dying inflammatory cells, and a result of the disintegrating membranes of these cells in long-standing lesions.¹⁷ Accumulation of cholesterol crystals can prevent healing in apical periodontitis lesions,¹⁷ but this accumulation does not seem to be associated with the maintenance of periapical cysts inasmuch as the frequency of its occurrence is low in these lesions.

In the present study, almost all periapical cysts presented stratified squamous epithelial lining, similarly to the findings of Lin *et al.*¹⁵ It is believed that epithelial status may be related to the growth of periapical cysts.¹⁸ The majority of the lesions evaluated in our study had atrophic epithelial lining, which was associated with moderate inflammatory infiltrate in the cystic capsule ($p < 0.01$), with a

diffuse location pattern ($p = 0.03$), and absence of neutrophils ($p = 0.01$). Nevertheless, according to Moreira *et al.*,¹⁸ no difference can be seen in the intensity of the infiltrate in lesions with atrophic or hyperplastic epithelium. These authors found different expression patterns of CD57, in which lesions with atrophic epithelium presented a higher percentage of CD57-positive cells. As the expression of CD57 is indicative of immunosuppression, it may constitute a negative immunomodulator of cystic growth.

Among the periapical lesions, periapical cysts presented the worst prognosis, and the larger lesions presented the worst evolution.¹⁹ It has been suggested that the tissues of periapical cysts are self-sustaining because they do not depend on the presence or absence of root canal infection. Therefore, because periapical cysts are less prone to heal after conventional endodontic therapy, surgical intervention is needed.¹³ For the treatment of periapical cysts, enucleation is the most commonly used technique,²⁰ although Carrillo *et al.*¹⁹ performed cyst

enucleation in combination with apicoectomy and retrograde filling. To achieve satisfactory periapical healing, surgical removal of a periapical cyst must include elimination of root canal infection.¹³

Conclusion

Periapical cysts with atrophic epithelial lining were related to moderate mononuclear inflammatory infiltrate in the cyst capsule. In addition, our findings demonstrated that the majority of the periapical cysts were asymptomatic, chronic lesions. It is imperative that the endodontist be well acquainted with the clinical, radiographic and microscopic features of periapical cysts in order to perform early diagnosis and establish proper treatment, thus increasing the success rates of endodontic therapy.

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