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"JÚLIO DE MESQUITA FILHO"
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# Histopathological and microbiological characterization of pulp tissue in severe (stage IV) periodontitis

Caracterização histopatológica e microbiológica do tecido pulpar na periodontite severa (estágio IV)

**Hévila de Figueiredo PIRES[1], Cíntia Mirela Guimarães NOBRE[1], Patrícia Guerra PEIXE[1], Ruthineia Diógenes Alves Uchôa LINS[1], Bruno César de Vasconcelos GURGEL**

1 - Universidade Federal do Rio Grande do Norte, Departamento de Odontologia. Natal, RN, Brazil.

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## ABSTRACT

**Objective:** To evaluate the morphology of the pulpal connective tissue and bacterial contamination in human teeth with advanced periodontitis. **Material and Methods:** Twenty-nine healthy teeth with severe periodontitis (stage IV periodontitis) and indication for extraction were collected. After dental extractions and histological preparation, tissue specimens were stained with hematoxylin and eosin, and Giemsa for microscopical analysis of the pulpal morphology and the presence of bacteria, respectively. **Results:** Several pulp tissue changes were observed in the 29 cases of severe periodontitis: degeneration of the odontoblastic layer (93.1%); dystrophic calcification (44.8%); fibrosis in the root (65.5%) and coronal (31%) regions; necrosis (20.7%), hyperemia (79.3%) and inflammatory infiltrate (82.8%), the latter being present in the root and coronal portions in 62.1% and 65.5% of cases, respectively. With regard to the degree of inflammatory infiltrate intensity, 48.3% had mild inflammatory infiltrate, 31% had moderate inflammatory infiltrate, and 3.4% had intense inflammatory infiltrate. Bacteria were detected in the pulp tissue in 31% of cases. No statistically significant associations were found between the morphological changes presented and the presence of inflammatory infiltrate and/or bacteria in the pulp tissue. **Conclusion:** Bacterial colonies are not always present in the pulp tissue of teeth with advanced periodontitis; however, this tissue usually presents inflammatory infiltrate, associated with several morphological alterations, of which odontoblast layer degeneration, hyperemia, and fibrosis should be highlighted. These findings underscore the importance of evaluating pulp status in teeth affected by severe periodontitis, supporting accurate diagnosis and interdisciplinary treatment planning in clinical practice.

## KEYWORDS

Bacterial infection; Dental pulp; Histology; Inflammatory infiltrate; Periodontal diseases.

## RESUMO

**Objetivo:** Avaliar a morfologia do tecido conjuntivo pulpar e a contaminação bacteriana em dentes humanos com periodontite avançada. **Material e Métodos:** Vinte e nove dentes hígidos com periodontite severa (periodontite estágio IV) e indicação de exodontia foram coletados. Após as extrações dentárias e preparo histológico, os espécimes teciduais foram corados com hematoxilina e eosina, e Giemsa, para análise microscópica da morfologia pulpar e da presença de bactérias, respectivamente. **Resultados:** Diversas alterações no tecido pulpar foram observadas nos 29 casos de periodontite severa: degeneração da camada de odontoblastos (93,1%); calcificações distróficas (44,8%); fibrose nas regiões radicular (65,5%) e coronária (31%); necrose (20,7%), hiperemia (79,3%) e infiltrado inflamatório (82,8%), sendo este último presente nas porções radicular e coronária em 62,1% e 65,5% dos casos, respectivamente. Com relação à intensidade do infiltrado inflamatório, 48,3% dos casos apresentaram infiltrado leve, 31% moderado e 3,4% intenso. Bactérias foram detectadas no tecido pulpar em 31% dos casos. Não foram encontradas associações estatisticamente significativas entre as alterações morfológicas observadas e a presença de infiltrado inflamatório e/ou bactérias no tecido pulpar. **Conclusão:** Colônias bacterianas nem

sempre estão presentes no tecido pulpar de dentes com periodontite avançada; contudo, esse tecido geralmente apresenta infiltrado inflamatório, associado a diversas alterações morfológicas, entre as quais se destacam a degeneração da camada de odontoblastos, hiperemia e fibrose. Esses achados ressaltam a importância de avaliar o estado pulpar em dentes acometidos por periodontite severa, contribuindo para um diagnóstico preciso e para o planejamento terapêutico interdisciplinar na prática clínica.

## PALAVRAS-CHAVE

Infecção bacteriana; Polpa dentária; Histologia; Infiltrado inflamatório; Doenças periodontais.

## INTRODUCTION

The relationship between periodontal tissues and the pulp can occur not only through anatomical connections (e.g. accessory canals, secondary canals, dentinal tubules, and apical foramen), but also through non-physiological pathways (e.g. iatrogenic perforations and periodontal pockets) that establish a functional relationship between them [1,2]. This intimate relationship may allow the pathological processes of one structure to influence another. It is believed that dental plaque on the root surface has the potential to cause pathological changes in the pulp, and that endodontic infection can affect the periodontal tissue; however, pulp collapse would not occur until the periodontal disease reaches the apical foramen [3,4]. Stage IV periodontitis represents the most advanced form of periodontal disease, characterized by severe attachment loss, tooth mobility, and complex treatment needs that require an interdisciplinary approach [5-7]. Epidemiological data indicate that Stage IV periodontitis affects a significant portion of the adult population, with prevalence rates ranging from 7% to over 11% worldwide [8], highlighting the clinical relevance and public health impact of this condition [9]. Understanding its effects on pulp tissue is essential for comprehensive patient management [1]. In 1974, Langeland et al. [10] studied the pulp cavity histology of 60 teeth that were free of caries but had different degrees of periodontitis. They demonstrated that histological changes might occur in the pulp tissues where periodontal disease is present; however, pulp death only occurs when the apical foramen is involved.

Classifications of endoperiodontal lesions have been modified over time to facilitate clinical diagnosis. The classifications made by Simon et al. [11] and Torabinejad and Kiger [12], although differing in their categorization, aimed to correctly clinical diagnose the disease, in relation to treatment. Currently, the classification proposed by the Academy of Periodontology and the European Federation of Periodontology, in 2018, includes the endoperiodontal involvement once again, evaluating acute or chronic events, and considering the presence or absence of endodontic and/or periodontal infections, and the history of trauma and iatrogenic factors [13,14]. With this classification, these data are used to assess the prognosis of the tooth and propose a treatment according to clinical signs, without generating questions regarding categorization.

Studies investigating the influence of periodontal disease on the pulp tissue are important to understand the evolution of the disease, the involvement of adjacent tissues and the effects of proposed treatments. The analysis of pulp changes could be of importance to determine the influence of advanced periodontal disease in the dental pulp, including its ability to cause, or not, inflammation or even pulp necrosis. From a clinical perspective, recognizing such changes can also assist in differentiating between endodontic, periodontal, and endo-periodontal lesions, thereby contributing to a more accurate diagnosis and appropriate treatment planning. Given the ongoing debate in the literature regarding the extent to which periodontal disease affects pulp vitality in the absence of direct bacterial contamination, this study was designed to help clarify this relationship. Thus, this study aims to analyze histologically the morphology of the conjunctive pulp tissue, as well as the presence of bacterial contamination, in teeth affected by severe periodontitis.

## MATERIAL AND METHODS

This descriptive and observational study was approved by the local Research Ethics Committee (CESMAC, 432/08). Twenty-nine single-rooted teeth with severe periodontitis, without caries and restorations, and suitable for extraction were collected from patients of both genders.

All volunteers signed a Free and Informed Consent Term. The criteria for tooth extraction should include at least two following reasons: oral rehabilitation purposes, tooth pain, tooth mobility, commitment of oral function, recurrent periodontal abscesses, and radiographically extensive bone loss. Probing pocket depth and clinical attachment level were also considered when these previous factors were present.

Criteria for inclusion were patients without systemic diseases, whose teeth were healthy, erupted, with complete root formation, pulp vitality, and free from caries or rehabilitation. All subjects were required to present severe periodontal disease, with periodontal pockets and bone loss extending to the apical portion of the root, clinical attachment loss, and the presence of marked mobility (stage IV). Anamnesis, physical examination of the oral cavity, and analysis of the periapical radiograph were performed before the surgical procedure for dental extraction. Dental and periodontal examinations were carried out by visualization of the dental recordings and periodontal chart. Probing pocket depth (mm) and clinical attachment level of the teeth were measured with a periodontal probe (North Carolina, Hu-Friedy, Chicago, IL, USA), and tooth mobility was measured with two metallic instruments [15]. Pulp sensibility tests (cold and hot sensitivities) were also performed to prove pulp vitality.

After extractions with local anesthesia, the collected teeth were immersed in 10% formaldehyde, demineralized with formic acid, dehydrated in an increasing ethanol chain (70 to 100%) and then embedded in paraffin. Longitudinally semi-serial sections were obtained with a microtome (thickness of 5 $\mu$m) and extended on glass slides that had been previously cleaned and degreased. Samples that presented the view of the whole pulp chamber and root canal in the specimens were chosen for analysis: two slides from each specimen were prepared for microscopic evaluation; one was stained with hematoxylin-eosin for the analysis of pulp morphology, and the other with Giemsa, to detect the presence of bacterial colonies inside the pulp. The microscopic analysis of each stained section was performed under the microscope at 100x magnification (Olympus CX31, Olympus Japan Co., Tokyo, JPN). A histological section of a human decayed tooth with pulp necrosis was used as a positive control for the Giemsa technique. This section was obtained from the Oral Pathology Laboratory of the University.

The pulp inflammation was categorized according to the localization: absent, present in the root portion of the pulp, and present in the coronal pulp. The pulp inflammation was also categorized according to its magnitude: absent, mild, moderate or intense. Two trained examiners performed this evaluation, based on the criteria proposed by Lins et al. [16], which included the intensity of the inflammatory infiltrate.

Descriptive and statistical analyses were performed using the SPSS (Statistical Package for the Social Sciences) for Windows version 21.0 (IBM Corp., Armonk, NY, USA). Fisher's exact test was used to investigate the association between the presence of inflammatory infiltrate, the morphological changes in the pulp tissue, and the presence of bacterial colonies. In addition, the test was used to investigate the association between age and the following parameters: fibrosis, calcification, and mobility grade II and III. For this purpose, the variable "age" was categorized into two groups: subjects aged $\leq 50$ years old and subjects aged $> 50$ years old. The level of significance was set at 5%.

## RESULTS

### Clinical analysis

The clinical analysis showed that the majority of patients were males (55.2%), aged from 32 to 70 years old and with a mean age of 52.21 ($\pm$8.78) years; the maxillary lateral incisor (tooth 22) and the lower central incisor (tooth 41) were the most affected teeth, with similar prevalence (22.6%) (Table I). Mobility level III was the most prevalent (51.7%), followed by mobility level II (37.9%), and the clinical attachment loss mean was 7.45 mm ($\pm$1.45). Additionally, the mean probing depth was 5.8 mm ($\pm$1.3), with minimum and maximum values of 2.8 mm and 9.3 mm, respectively (Table II).

### Histological and histochemical analysis

The morphological evaluation of the pulp of the 29 teeth included in this study revealed changes in the connective tissue, namely: odontoblast layer degeneration (Figure 1B) in 93.1% of cases (n = 27); dystrophic calcification in 44.8% of cases (n = 13); fibrosis in the root region in 65.5% of cases

**Table I -** Distribution of sample characteristics of teeth with periodontitis

| Sample characteristics | n | % |
|---|---|---|
| Age | 52.21 (±8.78)* | |
| Gender | | |
| Female | 13 | 44.8 |
| Male | 16 | 55.2 |
| Affected teeth | | |
| Upper central incisor | 1 | 3.4 |
| Upper lateral incisor | 6 | 22.6 |
| Upper canine | 3 | 10.3 |
| Upper premolar | 2 | 6.8 |
| Lower central incisor | 6 | 22.6 |
| Lower lateral incisor | 2 | 6.8 |
| Lower canine | 5 | 17.2 |
| Lower premolar | 3 | 10.3 |

*Mean (standard deviation).

**Table II -** Distribution of histological and clinical evaluation of teeth with periodontitis included in the study

| | Grading | Cases | Percentage |
|---|---|---|---|
| Probing Depth mean (mm) | (5.8 ± 1.3)* | | |
| Mobility | Grade I | 3 | 10.3% |
| | Grade II | 11 | 37.9% |
| | Grade III | 15 | 51.7% |
| Inflammation | No | 5 | 17.2% |
| | Yes | 24 | 82.8% |
| | Slight | 14 | 48.3% |
| | Moderate | 9 | 31.0% |
| | Severe | 1 | 3.50% |
| Hyperemia | No | 6 | 20.7% |
| | Yes | 23 | 79.3% |
| Necrosis | No | 23 | 79.3% |
| | Partial | 6 | 20.7% |
| Fibrosis | No | 11 | 37.9% |
| | Yes | 18 | 62.1% |
| Dental Layer Degradation | No | 2 | 6.90% |
| | Yes | 27 | 93.1% |
| Pulp stones | No | 16 | 55.2% |
| | Yes | 13 | 44.8% |
| Bacteria colony | No | 20 | 69.0% |
| | Root | 9 | 31.0% |
| | Coronal | 4 | 13.8% |

*Mean (standard deviation).

(n = 19) and also located in the coronal region in 31% of cases (n = 9). Areas of necrosis were observed in 20.7% of cases (n = 6) in two different regions: in the coronal region in 13.8% of cases (n = 4), and in the root region in 10.3% of cases (n = 3) (Figure 1C). Hyperemia was detected in 79.3% of cases (n = 23); in the coronal portion (65.5%; n = 19), and in the root portion (75.9%; n = 22). Pulp calcification was observed in 44.8% of cases (n = 13), and bacterial colonization was also detected in the root region (31.0%; n = 9) and in the coronal region (13.8%; n = 4) (Figure 1D)

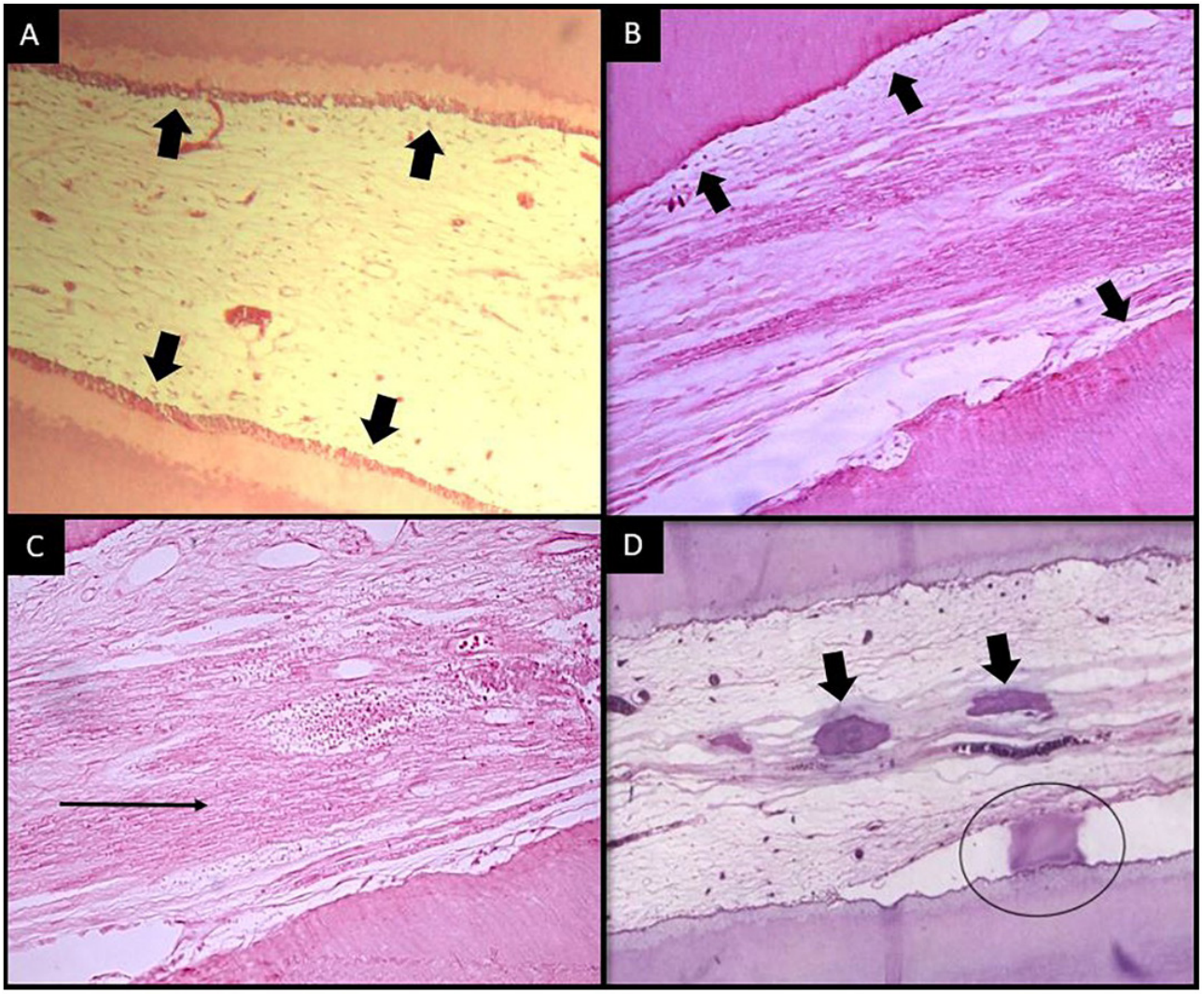


**Figure 1 -** Morphological evaluation of the pulp: (A) The black arrows point to the intact odontoblastic layer zone (H / E; 100X); (B) The black arrows show the degenerated odontoblastic layer (H / E; 100X); (C) The arrow shows the presence of pulpal tissue necrosis in the coronal third of the root (H / E; 100X); (D) The black arrow indicates zones of pulp calcification and the circle demarcates the presence of bacterial colonization (GIEMSA; 100X).

(Table II). Additionally, healthy areas with intact odontoblast layers were also visible in some areas of the histological specimens (Figure 1A).

An inflammatory infiltrate was present in 82.8% of cases (n = 24), located in the root portion of the pulp in 62.1% of cases (n = 18) and in the coronal pulp in 65.5% of cases (n = 19). With regard to the degree of inflammatory infiltrate intensity: 48.3% (n = 14) of teeth presented mild inflammatory infiltrate, 31% (n = 9) presented moderate inflammatory infiltrate and 3.4% (n = 1) had an intense inflammatory infiltrate. Only 17.2% (n = 5) demonstrated no inflammatory infiltrate. Giemsa staining detected the presence of bacteria in the root portion of the pulp tissue in 31% of the cases (n = 9). These colonies were also present in the coronal region of the pulp in 13.8% of the cases (n = 4) (Figure 1D) (Table III). No statistically significant differences ($p > 0.05$) were found between the morphological changes presented and the presence of inflammatory infiltrate (Table II) and/or bacteria in the pulp tissue (Table IV).

The cementum was found completely covering the root portion in all cases, being acellular and thinner in the coronal region, and cellular and thick towards the apical region, in association with areas of hypercementosis in the apical region. Areas with gaps or discontinuity were also observed, with cemental resorption, and the presence of remaining periodontal ligament lining some areas. Additionally, no statistically significant differences were found when analyzing the associations between the categorized age group and the following factors: mobility grade II and III ($p=0.672$), fibrosis ($p=0.411$), and calcification ($p=0.507$).

**Table III -** Associations between morphological changes in the pulp tissue and the presence of inflammatory infiltrate (Fisher's exact test)

| Events | | Inflammatory Infiltrate (cases/percentage) | | *p value* |
|---|---|---|---|---|
| | | Yes | No | |
| Hyperemia | Yes | 20 (69.0%) | 4 (13.8%) | 0.269 |
| | No | 3 (10.3%) | 2 (6.90%) | |
| Fibrosis | Yes | 15 (51.8%) | 9 (31.0%) | 0.644 |
| | No | 3 (10.3%) | 2 (6.90%) | |
| Calcification | Yes | 11 (37.9%) | 13 (44.9%) | 0.604 |
| | No | 2 (6.9%) | 3 (10.3%) | |
| Dental Layer Degradation | Yes | 23 (79.3%) | 1 (3.45%) | 0.320 |
| | No | 4 (13.8%) | 1 (3.45%) | |
| Necrosis | Yes | 4 (13.8%) | 20 (69.0%) | 0.269 |
| | No | 2 (6.9%) | 3 (10.3%) | |

**Table IV -** Association between the presence of bacterial colonies (shown by Giemsa staining) and the presence of inflammatory infiltrate (Fisher's exact test)

| | | Inflammatory Infiltrate | | *p* |
|---|---|---|---|---|
| | | Yes | No | |
| Bacteria colony | Yes | 7 (29.2%) | 17 (70.8%) | 0.502 |
| | No | 2 (40.0%) | 3 (69.0%) | |

## DISCUSSION

The results of this study showed that severe periodontitis can affect the morphology of pulp connective tissue. The presence of inflammatory infiltrate in the pulp tissue in 82.8% of cases of periodontitis showed that advanced periodontal disease could cause changes in adjacent tissues through different communication pathways, such as the apical foramen, and the lateral and accessory canals [17]. In the periodontal endodontic involvement, microorganisms could disseminate from one tissue to another and, therefore, interfere in their integrity [10,12].

The complexity of the canal system and its relationship with the periodontium allows bacteria and inflammatory mediators to circulate between the periodontium and the interior of the tooth [1,17]. Bacterial products, including enzymes, exotoxins, and endotoxins, and host cell inflammatory mediators, such as enzymes, cytokines, prostaglandins, and growth factors can morphologically alter the pulp tissue of teeth associated with periodontal disease [18]. Consistent with this idea, we observed that the inflammatory infiltrate, usually present in the pulp tissue, was associated with several morphological changes in the pulp, regardless of its intensity and the presence of bacterial colonies.

Concerning soft tissue organization, loss of pulp morphology, fibrosis, hyperemia, and degeneration of the odontoblast layer were frequent findings in this study. Corroborating these results, Fatemi et al. [19] carried out histological examinations of 20 hopeless permanent caries-free teeth from patients with periodontitis and found that 58.3% of teeth displayed edematous pulps, indicating an effect on the dental pulp. Inflammation, loss of odontoblast integrity, pulp with necrosis, and slight fibrosis were also observed. Assessing the pulp tissue of 242 teeth from 162 patients with moderate to severe periodontitis, Wan et al. [20] also found changes in the pulp tissue, with necrosis and fibrosis being the most frequent, followed by pulp inflammation, dystrophic calcifications, atrophy, vacuolization, and hyperemia. These authors concluded that the severity of periodontitis is significantly associated with histological changes in the pulp tissue. Gautam et al. [21] also reported similar morphological changes in a study that evaluated the pulps of 40 extracted teeth, associated with severe periodontitis. In agreement with Fatemi et al. [19] and Wan et al. [20], and the results of the current study, Gautam et al. [21] also concluded that there is a relationship between the progression of periodontal disease and the pulp changes found, which could be related to the

progression of the inflammatory process and the release of its mediators.

Dystrophic calcification present in some areas of tissue inflammation seems to be associated with cell degeneration and, therefore, the destruction of the cell membrane lipid bilayer, leading to the subsequent release of fatty acids, which eventually attract calcium ions to the region and promote calcification [22]. The areas of tissue hyperemia could be the consequence of the production of angiogenic factors, such as VEGF, PDGF, FGF-β, Angiopoietin, IL-1, IL-8, TNF-α, and IFN-γ. Moreover, the release of many of these growth factors and cytokines also stimulates fibroblast proliferation and collagen deposition, contributing to the formation of areas of fibrosis [2,23].

Pulp fibrosis may be related not only to the inflammatory process, but also to age and function [24]. Nemec et al. [25] analyzed 27 teeth extracted from dogs and suggested that changes in the pulp of teeth with advanced periodontal disease may reflect the mechanical injury caused by excessive tooth mobility. In this study, 31% of the cases presented tooth mobility grade II, and 62.1% presented grade III, which could also aggravate the changes observed in the pulp. According to Fatemi et al. [19] and Loya and Nikhade [26], fibrosis and calcification are more common findings in the pulps of elderly patients, although the pulp tissue of young patients may also present such changes due to external stimuli, regardless of age. In the present study, when age was categorized age into two groups, no significant associations were observed between age and degree of mobility, calcification or fibrosis. However, descriptive analysis showed that the subjects with a more advanced age (51 to 70 years old) presented fibrosis (61.1%), calcification (69.2%), mobility grade II (31.6%) and III (63.2%). On the other hand, subjects under 51 years old presented fewer cases of mobility grade II (30%) and III (60%), as well as fewer cases of fibrosis (38.9%) and calcifications (30.8%).

Rathod et al. [27] also performed a descriptive analysis of 20 periodontitis-extracted tooth pulps. An inflammatory infiltrate was found in most cases (85%), associated with the presence of pulp alterations, such as total and partial necrosis, fibrosis, loss of the odontoblastic layer, dystrophic calcification, hyperemia, and vascular dilatation [27]. These results are similar to our findings, although cases of complete pulp necrosis were not observed in the current investigation, only focal areas of necrosis were detected in some cases. Rathod et al. [27] suggested that the cases of necrosis observed could be due to bacterial contamination, as also observed in part of our sample, since the presence of bacteria in a sterile tissue such as the healthy pulp may induce tissue necrosis [27]. The presence of bacteria was observed mainly in the root pulp, in association with disorganization of the odontoblastic layer, necrosis, and hyperemia. However, no association between necrosis and inflammatory infiltrate, nor with bacterial colonization, were observed in the present study.

In cases of advanced periodontitis, the bacterial colonization in the pulp tissue occurs especially due to the presence of biofilm inside deep periodontal pockets, reaching the apical foramen [14,28,29]. This fact could justify the presence of bacterial colonies (identified by Giemsa staining), mainly in the root portion of the pulp tissue of the teeth (31% of cases), associated with advanced periodontitis, as examined in the present study. Giemsa stains can be used to identify the presence of bacteria and assess inflammatory status [30]. However, this colonization can also occur throughout the extensive root canal system, which is composed of numerous ramifications (subsequently assuming an ascending pattern), and less commonly in dentinal tubules [31].

Ricucci et al. [30] suggested that an intact cementum layer may protect the pulp from the effects of periodontal disease, but the pulp response varies depending on the extent of periodontal involvement. In our study, all teeth presented cementum that completely covered the root region, however, there were some areas of gaps or discontinuity with cemental resorption. Yu and Abbott [31] suggested that areas of gaps or discontinuity with cemental resorption leave the dentin tubules exposed, facilitating pulp infection. Torabinejad and Kiger [12] and Galler et al. [32] proposed that the pulp displays a good defense capability when the blood supply via the apical foramen remains intact, and that pulp death does not occur until there is involvement of the apical foramen in the bacterial biofilm.

This study provides evidence that inflammatory changes in the pulp tissue may occur due to the advanced stage of periodontal disease. To date, there has been no definitive conclusion regarding the role of periodontal disease in pulp tissues. The

major limitations of our study include the lack of uniform documentation of case histories, and failure to include dental control pulps with normal periodontium [20]. Other pulp stains could be used to identify bacterial colonies in the pulp, such as Brenn and Brown staining; however, Giemsa can also identify nuclei, cytoplasm, and bacteria [33]. Giemsa staining exhibits lower sensitivity when compared to more advanced molecular techniques, such as PCR, immunohistochemistry, and fluorescence in situ hybridization (FISH) [34,35], which could be employed in future studies to provide a more comprehensive analysis of pulp response to periodontal disease. Additional limitations include the small sample size and potential selection bias, as the teeth indicated for extraction may represent particularly severe cases, already exhibiting histological alterations due to infectious, inflammatory, and/or mechanical injuries.

Furthermore, well-controlled studies should be performed to better understand the relationship between advanced periodontal disease and pulp inflammation, including the comparison of the pulp morphology of teeth with periodontitis, under less severe conditions; although the provision of suitable control groups for this type of study is not easy. Ideally, teeth from the same subject should serve as the control and test group, but it is uncommon for both hopeless healthy and severe periodontitis teeth extractions to occur. From a clinical standpoint, the identification of pulpal alterations associated with advanced periodontitis emphasizes the importance of conducting pulp sensibility tests to assess tissue vitality in compromised teeth. In the future, the application of molecular techniques such as PCR, immunohistochemistry, and FISH may enhance understanding of bacterial presence and pulpal inflammatory response, thereby complementing findings obtained through conventional histological methods like Giemsa staining.

## CONCLUSIONS

In conclusion, severe periodontitis is frequently associated with histological alterations in the pulp tissue, including inflammatory infiltrate, degeneration of the odontoblast layer, hyperemia, and fibrosis. These changes were observed even in the absence of bacterial colonies, suggesting that non-bacterial mechanisms, such as inflammatory mediators, may play a significant role in pulpal alterations. These findings highlight the importance of careful clinical evaluation, including pulp sensibility testing, in teeth with periodontal involvement. An interdisciplinary diagnostic and therapeutic approach are essential to ensure appropriate management and improve clinical outcomes in such complex cases.

## Author's Contributions

HFP: Data Curation, Formal Analysis, Writing – Original Draft Preparation. CMGN: Methodology, Investigation, Data Curation, Formal Analysis, Writing – Original Draft Preparation. PGP: Methodology, Investigation, Data Curation, Formal Analysis, Writing – Original Draft Preparation. RDAUL: Methodology, Formal Analysis, Validation, Writing – Review & Editing, Supervision. BCVG: Conceptualization, Methodology, Investigation, Formal Analysis, Validation, Writing – Review & Editing, Supervision.

## Conflict of Interest

No conflicts of interest declared concerning the publication of this article.

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## Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subject's oversight committee guidelines and policies. This study protocol was reviewed and approved by The Committee for Ethics in Research (CEP) of CESMAC approved this study approval number (432/08).

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**Hévila de Figueiredo Pires**
**(Corresponding address)**
Universidade Federal do Rio Grande do Norte, Departamento de Odontologia, Natal, RN, Brazil.
Email: hevilapires@gmail.com

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