Original Research
Oral Pathology

Evaluation of the expression of nerve fiber markers in healthy and inflamed dental pulp

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Declaration of Interests: The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

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https://doi.org/10.1590/1807-3107bor-2023.vol37.0020

Submitted: October 17, 2021
Accepted for publication: September 19, 2022
Last revision: September 30, 2022

Abstract: The diagnosis of irreversible pulpitis (IP) depends on clinical data, especially the chief complaint of the patient, visual inspection, response to the application of stimuli, and radiographic examination. The characterization of nerve fibers (NF) in IP may contribute to better interpret painful symptoms, but has been barely explored. This study sought to characterize the density and integrity of NF in 16 samples of IP and in five healthy pulps (HP) using S-100 and PGP 9.5 markers. Immunohistochemistry was performed to determine the density/mm^2 of S-100$^+$ and PGP 9.5$^+$ in NF. The amount of degenerated NF was obtained by subtracting the total NF density from the amount of intact NF. Associations between NF density and integrity and symptomatology were calculated. All samples were positive for S-100 and PGP 9.5. Compared to HP samples (38.20/mm^2), IP samples had a lower density of intact NF (6.24/mm^2). A significantly higher density of degenerated NF was found in IP samples with spontaneous pain (39.59/mm^2) compared to those with provoked pain (23.96/mm^2) ($p = 0.02$). No association was observed between intensity of the inflammatory infiltrate and NF density and integrity ($p > 0.05$). The findings of this study suggest that pulpitis may involve different stages of degeneration and may be more advanced in cases with spontaneous pain. The symptoms reported by affected individuals do not appear to depend on the intensity of the inflammatory infiltrate, but rather on the integrity of NF.

Keywords: Dental Pulp; Immunohistochemistry; Nerve Fibers.

Introduction

The dental pulp is a complex connective tissue that, together with the periodontal ligament, is highly innervated with trigeminal sensory neurons and a high nociceptor density.[1] The pulpodentine complex is densely innervated and pain occurs when this tissue is attacked or inflamed.[2,3] The sensory innervation of the dental pulp occurs through axons originating from trigeminal nodules, whose nerve fibers (NF) are mainly type A and C.[4-7] Type A myelinic fibers are responsible for rapid responses to acute painful stimuli and are located in the outermost portion of the coronary pulp. Type C unmyelinated fibers are responsible with a slower transmission of painful stimuli in the presence of aggression.[4,5,7,8]

Dental caries is the main aggression that affects pulp tissue, and its progression triggers an inflammatory process that alters the physiology and integrity of the NF, influencing the pain threshold of the affected individual.[9,10]

Pain assessment based on the response to dental pulp sensibility tests is currently the clinical tool most commonly used to determine pulp status and severity of inflammation and to suggest the diagnosis.[3,11] However, a systematic review has revealed that the characterization of pain based only on clinical tests does not appear to be entirely reliable for making an endodontic diagnosis.[12] In this respect, different classification systems have been reported for pulpal diagnosis, but most of them combine clinical and histological criteria that can result in diagnostic confusion.[13-16] This is due to the fact the histopathological data of the inflamed pulp are not available during the clinical examination and that the association between histology and painful symptoms is not clear.[12,13,16,17] Regarding pulp changes, irreversible pulpitis (IP) occurs more frequently in decayed posterior teeth or in extensive restorations with pulp involvement in female individuals.[12]

Although it is not the only criterion for the definition of IP, provoked pain that persists after the stimulus is removed or spontaneous pain, in addition to a positive response to dental pulp sensibility tests, is the main clinical finding.[5,13,14,18] Previous studies have demonstrated a possible association between pain and changes in the dental pulp of IP; however, this link has not yet been fully elucidated.[12] Specific markers for neuronal proteins such as S-100 and PGP 9.5 have been used to evaluate nerve structures in the dental pulp.[18-21] The S-100 protein, expressed mainly by Schwann cells, has been used to investigate NF density in healthy and inflamed human pulp,[18,22] and NF integrity is possibly impaired in an inflamed pulp with the evolution of inflammation. In addition, the protein gene product 9.5 (PGP 9.5) is a neuron-specific protein with opposite biologic role, functioning as a ubiquitin carboxyl-terminal hydrolase and ligase in which it is expressed in axons and used to map patients with neurodegenerative conditions.[23,24] Importantly, PGP 9.5 has also been used to quantify the neural protein recovered from pulpal tissue.[20] The hypothesis is that the early loss or absence of expression of this protein appears to be associated with nerve degeneration processes.[23,24] Thus, PGP 9.5 expression can be a promising marker for the identification of NF in inflamed dental pulp.

According to Diogenes,[25] innervation is a key component of the pulpodentine complex, since it modulates vascular, immunological, and dentinogenic responses to injuries in addition to having a sophisticated sensory function. Given the high frequency of IP, the diagnosis and treatment of which are based mainly on clinical data, including painful symptoms,[14,19] and the scarcity of investigations that have documented the association between symptoms and histopathological features of pulp innervation,[21,26] the microscopic characterization of pulpal NF status may contribute to a better interpretation of the pain experienced by the patient. In view of the above considerations, the purpose of the present study was to analyze the density and integrity of NF in samples of inflamed human dental pulp with a clinical diagnosis of IP and in healthy pulp (HP) based on the immunoexpression of the S-100 and PGP 9.5 markers.

Methodology

Study design and dental pulp samples

A total of 21 human teeth extracted for endodontic, periodontal, or surgical reasons in the Dental Urgency Service of the School of Dentistry, Universidade Federal de Goiás, Goiânia, Brazil were selected for this study as a convenience sample, as used by other authors in similar researches.[17,20] Sixteen of these teeth had a clinical diagnosis of inflamed pulp based on the following criteria: spontaneous symptomatology, positive response to a pulp sensibility test (1,1,1,2-tetrafluoroethane spray; Endo-Ice, Maquira, Paraná, Brazil), and absence of pulp exposure associated with dental caries. Five teeth, intact impacted third molars with indication for extraction, had the dental pulp removed and used as controls. The patients had no history of systemic diseases, nor had they

used any medications (e.g., anti-inflammatory drugs) during the last three months. Also, the tooth couldn't have undergone previous restorative treatment or have clinicoradiographic findings of periapical disease.[17] The study was approved by the local Ethics Committee (#102/2007). Patients' identities remained anonymous according to the Declaration of Helsinki.

All 16 teeth with inflamed pulp were properly dried and isolated before the dental pulp sensibility test. Of these teeth, two were extracted due to associated significant periodontal bone loss and fixed in 10% formaldehyde. The other teeth were treated following the protocol: anesthesia, absolute isolation of the operative field, asepsis with 1% sodium hypochlorite, and coronary opening. After determining the working length, the dental pulp was removed with Hedström files and gently placed on filter paper for fixation in 10% formaldehyde to aid in specimen positioning during histological processing. The root canal was then prepared and filled using a lateral condensation technique with gutta-percha points and an endodontic sealer.

Clinicopathologic data

Demographic data (age and sex), affected tooth, symptomatology, i.e., reported spontaneous or provoked pain that lingered, pain on palpation (absent or present), pulp test results (negative or positive), and horizontal percussion test (negative or positive) were obtained for each patient. The extracted teeth included in the study were decalcified for 90 days in ethylenediamine tetra acetic acid (EDTA). Five-micrometers-thick sections of paraffin-embedded material obtained from each case were stained with hematoxylin and eosin (H&E). The degree of deposition of collagen fibers, the intensity of the inflammatory infiltrate, areas of exudate, vascular congestion, and state of organization of the odontoblastic layer were evaluated. Cases with intense collagen deposition were excluded from the study.

Immunohistochemistry

In each case, 3-µm-thick sections mounted on polarized slides were subjected to immunohistochemical analysis by the polymer-based detection method. Immunohistochemical analyses were performed using monoclonal antibodies against anti-S-100 (clone N1573; Dako; 1:1000) and anti-PGP 9.5 (clone Z5116, Dako; 1:300). For the deparaffinization, rehydration and antigen-retrieval steps, the sections were submitted to TRILOGY™ Concentrate (Cell Marque; 1:100) at 96°C in a digital water bath (DeLeo) for 30 minutes. Next, the sections were treated with the Novolink™ Max Polymer Detection System (Novocastra, Leica Biosystems Gmb) and the reactions were developed with 3.3'-diaminobenzidine (DAB; Dako). S-100$^+$ or PGP 9.5$^+$ NF was considered as an internal positive control, while negative controls consisted of replacement of the primary antibody with 1% bovine serum albumin in the buffer solution.

Microscopic evaluation

H&E-stained slides were subjected to quantitative analysis of the inflammatory infiltrate in 10 alternating microscopic fields at ×40 magnification. The results of the histological analysis were defined according to the method of Bruno et al.[17] and Vaz et al.[27], *i.e.*, a) without inflammation, when most of the fields evaluated (> 7) did not have inflammatory cells; b) mild inflammation, when most fields (> 7) had < 35% of inflammatory cells; and c) intense inflammation, when most fields (> 7) had ≥ 35% of inflammatory cells. All teeth had dental caries. The presence of exudate areas, disorganization of the odontoblastic layer, and congested vessels was also investigated.

S-100$^+$ and PGP 9.5$^+$ NF were evaluated quantitatively (NF$^+$ by density/mm^2). The assessment was performed on the entire pulp tissue (*i.e.*, coronary and root area). All sections were evaluated by two examiners (E.F.M. and D.A.C.A.) in a blinded fashion. Disagreements were jointly reviewed in order to reach a consensus. Briefly, NF was quantified in each case (S-100$^+$ and PGP 9.5$^+$) and mean density in mm^2 was obtained using the area determined by an integration reticle (4740680000000-Netzmikrometer ×12.5; Carl Zeiss, Göttingen, Niedersachsen, Germany) connected to a light microscope (AxioScope; Carl Zeiss, Niedersachsen, Germany). The quantification was performed in 10 alternating fields at ×40 magnification. At this magnification, the area of one

field corresponds to 0.0961 mm^2. Then, the amount of NF PGP 9.5 (intact) was subtracted from the value of S-100 (intact and degenerated) of each case in order to obtain the number of degenerated NF.

Data analysis

Data were analyzed using the GraphPad Prism software version 7.00 (GraphPad Software, San Diego, USA). The Shapiro-Wilk test revealed a non-normal distribution of the data. A non-parametric Mann-Whitney test was used for comparative analysis of NF density and inflammatory infiltrate. The level of significance was set at < 0.05 in all analyses.

Results

A total of 16 teeth with a diagnosis of IP were selected, 11 (68.8%) maxillary molars and five (31.2%) maxillary incisors. The teeth were extracted due to endodontic (n = 4), periodontal (n = 4), or surgical (n = 8) reasons. The mean age of the individuals was 31.2 (± 11.6) years (range: 17–65 years). All of them had a positive pulp sensibility test to cold and a closed cavity due to carious lesion, 10 (62.5%) of them reported spontaneous pain, and six (37.5%) reported provoked pain. For the HP group, five maxillary molars of individuals with a mean age of 28.3 (± 6.3) years, most of them men (n =4 ; 81.6%), were included.

In all cases of IP, mild collagen deposition, areas of exudate, vessel congestion, disorganization or degeneration of the odontoblastic layer, and a predominantly mononuclear inflammatory infiltrate were observed (Figure 1A and B). Thirteen (81.3%) cases had a mild inflammatory infiltrate, while three (18.7%) cases exhibited an intense inflammatory filtrate. The five control teeth (intact

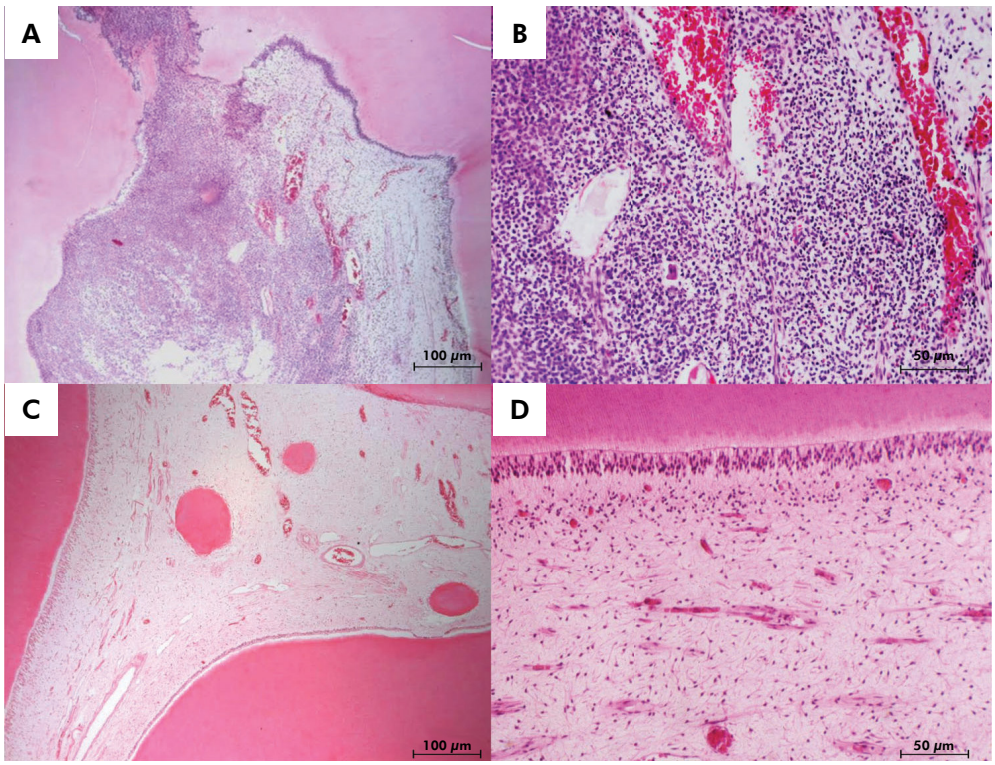

Figure 1. (A, B) Morphological characteristics detected in irreversible pulpitis samples. There are areas of disorganization in the odontoblastic layer, congested vessels, and intense, predominantly mononuclear inflammatory infiltrate. (C, D) Morphological characteristics observed in healthy pulps samples. Preserved odontoblastic layer, possible calcifications, and no signs of inflammation are present (hematoxylin and eosin, ×20 and ×40, respectively).

teeth) had normal microscopic pulp architecture with normally aligned odontoblasts and absence of inflammatory cells, dilated vessels, or fibrosis (Figure 1C and D).

All samples were positive for S-100 and PGP 9.5. The brown labeling of these proteins was evident in the nuclear and cytoplasmic region of the NF (Figure 2). Regarding the clinical characteristics (age, sex, tooth, referred pain, pain on palpation, horizontal percussion test) of IP and their association with immunoexpression of S-100^{+} and PGP 9.5^{+} in the analysis of the density and integrity of the NF, no significant association was observed ($p > 0.05$). IP samples showed a lower density of intact NF regarding the expression of PGP 9.5^{+} (6.24/mm^2) compared to control samples (38.20/mm^2) ($p = 0.01$) (Figure 3A). In addition, a significantly higher density of degenerated NF was found in IP samples with spontaneous pain (39.59/mm^2) than in samples with provoked pain (23.96/mm^2) ($p = 0.02$) (Figure 3B). No association was observed between the density and integrity of NF and the intensity of the inflammatory infiltrate ($p > 0.05$).

Discussion

Pain is the only sensation induced in response to activation of pulp sensory nerves, regardless of the type of stimulus applied to the tooth. The groups of pulp nerve fibers may explain changes in the quality of symptoms in pulp inflammation. The type and duration of symptoms in teeth with pulp inflammation are of diagnostic value and can give some indication of the type of pulp disease.[12,14] It should be stressed that the poor correlation between clinical symptoms and histopathological changes in pulpitis and the determination of the type and extent of inflammatory changes based

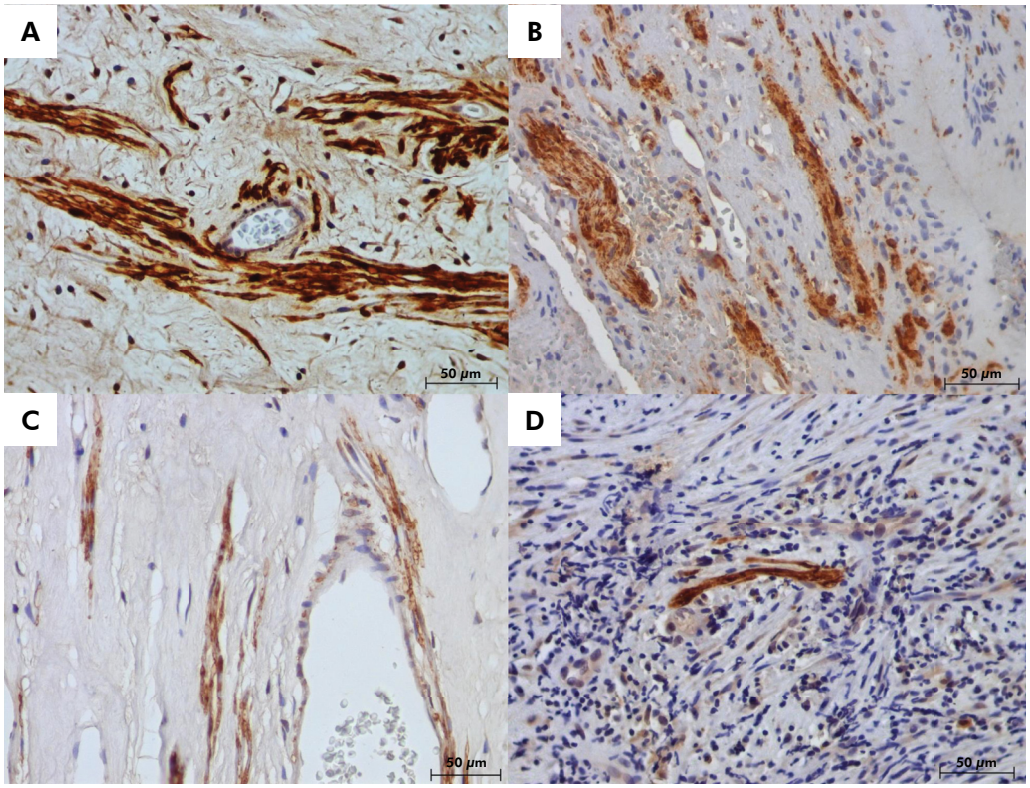

Figure 2. Similar density of S-100^{+} (A, B) and reduced density of nerve fibers in PGP 9.5^{+} expression **(C, D)** in irreversible pulpitis samples (B, D) compared to healthy pulp samples (A, C) (Immunohistochemistry, ×40).

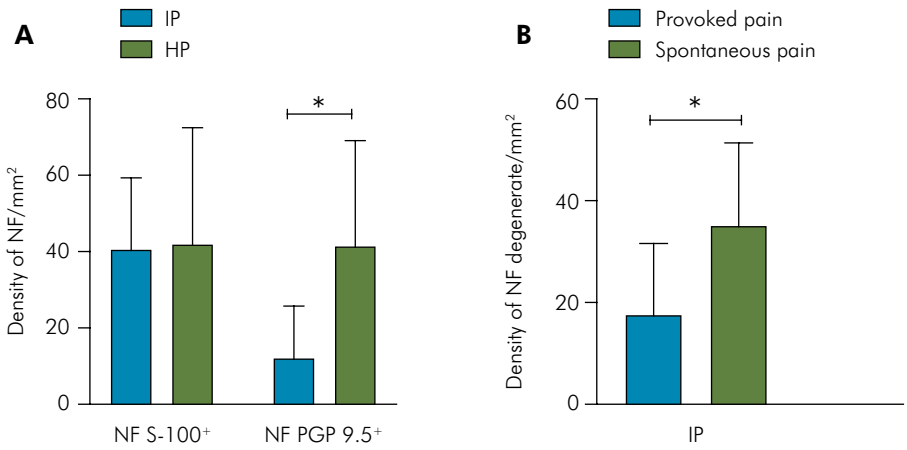

Figure 3. (A) Density of nerve fibers (NF) expressing S-100⁺ and PGP 9.5⁺ in irreversible pulpitis (IP) and in healthy pulp (HP) samples. (B) Density of degenerated NF in IP samples with reported information about spontaneous and provoked pain.

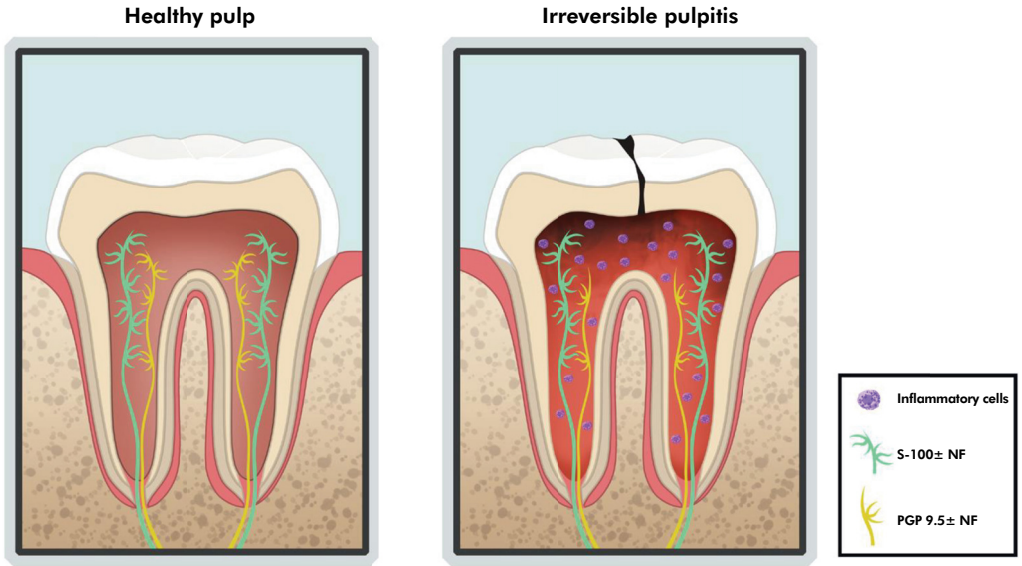

Figure 4. Schematic figure illustrating the density of nerve fibers (NF). S-100⁺ similarities for irreversible pulpitis (IP) and healthy pulp, while there is a pronounced reduction of intact NF by PGN 9.5⁺ in IP.

on symptomatology is inaccurate.[2,3,28] On this basis, in the inflamed dental pulp, the terminals of the afferent primary nociceptors detect the presence of mediators with receptors that are synthesized in the cell body of the afferent fiber and then transported to the periphery. If the mediator reaches a sufficient concentration in the inflamed tissue to activate the receptor, the nociceptive neuron can be activated, *i.e.*, the membrane would be conducted to the central nervous system or sensitized.[3,25] Thus, attention has been closely paid to toothache, described as pain originating from innervated dental tissue or tissues immediately adjacent to the teeth, since it is a common experience and is considered to be a public health concern.[29] In view of the high occurrence of IP,[14,19] it is essential to understand the role of neural markers in order to elucidate and mitigate the painful symptoms that often occur in affected individuals.

This study revealed a similar density of NF by immunoexpression of S-100$^+$ for IP and HP, as well as a reduction of intact NF by PGN 9.5$^+$ in the IP samples, as illustrated in Figure 4. Also, a higher density of degenerated NF was demonstrated in IP samples associated with spontaneous pain. These findings suggest that the evolution of inflammation may alter the axonal integrity of dental pulp NF, promoting pain conditions regardless of the presence of a stimulus. Accordingly, previous studies have used S-100 protein to identify NF in inflamed and healthy pulp.[18,21] A study has detected a higher density of NF by immunoexpression of S-100B$^+$ in inflamed pulp associated with spontaneous pain than in HP samples. Although the quantification methods were not described, the authors argued that inflammation may induce NF ramifications by increasing their quantity.[18] Likewise, a higher density of S-100$^+$ structures was reported in inflamed pulps than in the control group. The authors postulated that this high density was associated with the expression of this protein in the NF, macrophages, and dendritic cells during inflammation.[21] Conversely, our study carried out a comparative evaluation of NF by the expression of S-100$^+$ in IP and HP by quantifying in a standardized manner only those structures with NF morphology that were degenerated or intact.

PGP 9.5 has been employed to assess axonal integrity, with reduced expression in NF altered by neurodegenerative diseases[23,24] and leprosy.[30,31] Nevertheless, the use of this marker has also been considered to identify NF in inflamed pulp.[20,32,33] The expression of PGP 9.5$^+$ in intact NF of inflamed pulp and HP was demonstrated by an immunofluorescence assay.[33] Additionally, a study revealed a similar expression of PGP 9.5$^+$ in inflamed healthy pulp by using a western blot assay. Certainly, this similarity occurred because the evaluation of PGP 9.5 expression was performed in nerve structures and cells with neural receptors.[20] Rodd and Boissonade[32] evaluated the double expression of PGP 9.5 and substance P by indirect immunofluorescence in symptomatic and asymptomatic pulp. The authors, however, found a greater expression of PGP 9.5/substance P$^+$ in NF samples associated with pain. This is in line with our findings, which revealed a relationship between the expression of PGP 9.5, the state of integrity of pulp NF and the symptomatology, *i.e.*, provoked or spontaneous pain.

Herein, the difference in PGP 9.5$^+$ and S-100$^+$ expression in intact NF resulted in the amount of degenerated NF, whose density was higher in samples with spontaneous pain. Furthermore, no association was detected between the density of degenerated NF and the intensity of the inflammatory process. In fact, the evolution of inflammation may lead to different stages of NF degeneration, particularly in type C fibers that are more resistant to aggression.[5,9,10] In addition to inflammatory cells, vascular events and neuromodulatory mediators secreted in the presence of an aggression increase the volume of pulp tissue, promoting NF compression and axonal degeneration.[34,35] However, the increase in inflammatory mediators may modify the sensory ion channels and depolarize the plasma membrane of the NF. In principle, this change may increase the susceptibility of the NF to triggering action potentials in the absence of any stimulus.[36] Although spontaneous pain is poorly understood, possibly the most advanced stage of degeneration of pulp NF is one of the factors responsible for the excitability of these structures. Considering the link between higher density of degenerated NF and spontaneous pain, the report of this symptom may be another important clinical parameter for the therapeutic decision of pulpectomy; however, these findings should be further explored in a large survey.

Similar to the data reported in the literature,[14,36] our IP samples were mainly from decayed molars. Our results highlight the fact that understanding the pathophysiology of pulp status can mitigate the burden on the public health system. Nonetheless, we acknowledge that this study reports preliminary results based on a limited convenience sample, a fact that may be inherent to screening of oral health services, which explains the difficulty in obtaining IP samples. Future studies should standardize patients by age group to address the hypothesis that age may influence the alteration of NF density in patients with IP.

Conclusion

In summary, a lower density of NF was found in teeth diagnosed with IP than in those diagnosed with HP, whereas the density of degenerated NF was higher in cases with spontaneous pain. These data suggest that the pulp tissue may be in different degeneration stages in cases of IP, being more advanced in cases with spontaneous pain. Thus, the referred pain can certainly contribute to the therapeutic decision of the clinician and endodontist regarding pulpectomy.

Acknowledgements

This study is dedicated to the memory of Professor Aline Carvalho Batista, who developed the method and sadly passed away prematurely. This study was supported in part by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil. C.E. and E.F.M. are research fellows of CNPq. We also thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brazil (Capes, finance code 001). J.A.A.A. is the recipient of fellowship. Mrs. E. Greene provided English editing of the manuscript.

References

1. Henry MA, Hargreaves KM. Peripheral mechanisms of odontogenic pain. Dent Clin North Am. 2007 Jan;51(1):19-44. https://doi.org/10.1016/j.cden.2006.09.007
2. Byers MR, Taylor PE, Khayat BG, Kimberly CL. Effects of injury and inflammation on pulpal and periapical nerves. J Endod. 1990 Feb;16(2):78-84. https://doi.org/10.1016/S0099-2399(06)81568-2
3. Galler KM, Weber M, Korkmaz Y, Widbiller M, Feuerer M. Inflammatory response mechanisms of the dentine-pulp complex and the periapical tissues. Int J Mol Sci. 2021 Feb;22(3):1480. https://doi.org/10.3390/ijms22031480
4. Bender IB. Pulpal pain diagnosis: a review. J Endod. 2000 Mar;26(3):175-9. https://doi.org/10.1097/00004770-200003000-00012
5. Abd-Elmeguid A, Yu DC. Dental pulp neurophysiology: part 1. Clinical and diagnostic implications. J Can Dent Assoc. 2009 Feb;75(1):55-9.
6. Levin LG, Law AS, Holland GR, Abbott PV, Roda RS. Identify and define all diagnostic terms for pulpal health and disease states. J Endod. 2009 Dec;35(12):1645-57. https://doi.org/10.1016/j.joen.2009.09.032
7. Donnelly CR, Shah AA, Suh EB, Pierchala BA. Ret signaling is required for tooth pulp innervation during organogenesis. J Dent Res. 2019 Jun;98(6):705-12. https://doi.org/10.1177/0022034519837971
8. Torebjörk HE, Hallin RG. Perceptual changes accompanying controlled preferential blocking of A and C fibre responses in intact human skin nerves. Exp Brain Res. 1973 Jan;16(3):321-32. https://doi.org/10.1007/BF00233334
9. Kassebaum NJ, Bernabé E, Dahiya M, Bhandari B, Murray CJ, Marcenes W. Global burden of untreated caries: a systematic review and metaregression. J Dent Res. 2015 May;94(5):650-8. https://doi.org/10.1177/0022034515573272
10. Conrads G, About I. Pathophysiology of dental caries. Monogr Oral Sci. 2018;27:1-10. https://doi.org/10.1159/000487826
11. Jespersen JJ, Hellstein J, Williamson A, Johnson WT, Qian F. Evaluation of dental pulp sensibility tests in a clinical setting. J Endod. 2014 Mar;40(3):351-4. https://doi.org/10.1016/j.joen.2013.11.009
12. Zanini M, Meyer E, Simon S. Pulp inflammation diagnosis from clinical to inflammatory mediators: a systematic review. J Endod. 2017 Jul;43(7):1033-51. https://doi.org/10.1016/j.joen.2017.02.009
13. Endodontics: colleagues for excellence. Chicago: American Association of Endodontists; 2013 [cited 2021 Apr 7]. Available from: https://www.aae.org/specialty/wp-content/uploads/sites/2/2017/07/endodonticdiagnosisfall2013.pdf
14. Estrela C, Guedes OA, Silva JA, Leles CR, Estrela CR, Pécora JD. Diagnostic and clinical factors associated with pulpal and periapical pain. Braz Dent J. 2011;22(4):306-11. https://doi.org/10.1590/S0103-64402011000400008
15. Abbott PV, Yu C. A clinical classification of the status of the pulp and the root canal system. Aust Dent J. 2007 Mar;52(1 Suppl):S17-31. https://doi.org/10.1111/j.1834-7819.2007.tb00522.x
16. Dummer PM, Hicks R, Huws D. Clinical signs and symptoms in pulp disease. Int Endod J. 1980 Jan;13(1):27-35. https://doi.org/10.1111/j.1365-2591.1980.tb00834.x
17. Bruno KF, Silva JA, Silva TA, Batista AC, Alencar AH, Estrela C. Characterization of inflammatory cell infiltrate in human dental pulpitis. Int Endod J. 2010 Nov;43(11):1013-21. https://doi.org/10.1111/j.1365-2591.2010.01757.x
18. Dourou V, Lyroudia K, Karayannopoulou G, Papadimitriou C, Molyvdas I. Comparative evaluation of neural tissue antigens:neurofilament protein (NF), peripherin (PRP), S100B protein (S100B), neuron-specific enolase (NSE) and chromogranin-A (CgA)—in both normal and inflamed human mature dental pulp. Acta Histochem. 2006;108(5):343-50. https://doi.org/10.1016/j.acthis.2006.06.001

19. Lin PF, Fiore-Donno G, Lombardi T. Immunohistochemical detection of S-100 protein in human deciduous dental pulp. Ann Anat. 1994 Apr;176(2):171-3. https://doi.org/10.1016/S0940-9602(11)80444-9
20. Warren CA, Mok L, Gordon S, Fouad AF, Gold MS. Quantification of neural protein in extirpated tooth pulp. J Endod. 2008 Jan;34(1):7-10. https://doi.org/10.1016/j.joen.2007.09.014
21. Manolea H, Vasile N, Opri M, Fronie A, Popescu MR. Immunohistochemical and electron microscopy aspects of the nerve structures from the dental pulp. Rom J Morphol Embryol. 2014;55(1):147-52.
22. Gonzalez-Martinez T, Perez-Piñera P, Díaz-Esnal B, Vega JA. S-100 proteins in the human peripheral nervous system. Microsc Res Tech. 2003 Apr;60(6):633-8. https://doi.org/10.1002/jemt.10304
23. Chiang HY, Huang IT, Chen WP, Chien HF, Shun CT, Chang YC, et al. Regional difference in epidermal thinning after skin denervation. Exp Neurol. 1998 Nov;154(1):137-45. https://doi.org/10.1006/exnr.1998.6896
24. Bilguvar K, Tyagi NK, Ozkara C, Tuysuz B, Bakircioglu M, Choi M, et al. Recessive loss of function of the neuronal ubiquitin hydrolase UCHL1 leads to early-onset progressive neurodegeneration. Proc Natl Acad Sci USA. 2013 Feb;110(9):3489-94. https://doi.org/10.1073/pnas.1222732110
25. Diogenes A. Trigeminal sensory neurons and pulp regeneration. J Endod. 2020 Sep;46(9 9S):S71-80. https://doi.org/10.1016/j.joen.2020.06.038
26. England MC, Pellis EG, Michanowicz AE. Histopathologic study of the effect of pulpal disease upon nerve fibers of the human dental pulp. Oral Surg Oral Med Oral Pathol. 1974 Nov;38(5):783-90. https://doi.org/10.1016/0030-4220(74)90401-0
27. Vaz MM, Lopes LG, Cardoso PC, Souza JB, Batista AC, Costa NL, et al. Inflammatory response of human dental pulp to at-home and in-office tooth bleaching. J Appl Oral Sci. 2016 Sep-Oct;24(5):509-17. https://doi.org/10.1590/1678-775720160137
28. Närhi MV. The characteristics of intradental sensory units and their responses to stimulation. J Dent Res. 1985 Apr;64(Spec No):564-71. https://doi.org/10.1177/00220345850640041l
29. Barasuol JC, Santos PS, Moccelini BS, Magno MB, Bolan M, Martins-Júnior PA, et al. Association between dental pain and oral health-related quality of life in children and adolescents: A systematic review and meta-analysis. Community Dent Oral Epidemiol. 2020 Aug;48(4):257-63. https://doi.org/10.1111/cdoe.12535
30. Ebenezer GJ, Daniel E. Expression of protein gene product 9.5 in lepromatous eyes showing ciliary body nerve damage and a "dying back" phenomenon in the posterior ciliary nerves. Br J Ophthalmol. 2004 Feb;88(2):178-81. https://doi.org/10.1136/bjo.2003.027276
31. Antunes SL, Chimelli LM, Rabello ET, Valentim VC, Corte-Real S, Sarno EN, et al. An immunohistochemical, clinical and electroneuromyographic correlative study of the neural markers in the neuritic form of leprosy. Braz J Med Biol Res. 2006 Aug;39(8):1071-81. https://doi.org/10.1590/S0100-879X2006000800010
32. Rodd HD, Boissonade FM. Substance P expression in human tooth pulp in relation to caries and pain experience. Eur J Oral Sci. 2000 Dec;108(6):467-74. https://doi.org/10.1034/j.1600-0722.2000.00924.x
33. Alvarado LT, Perry GM, Hargreaves KM, Henry MA. TRPM8 Axonal expression is decreased in painful human teeth with irreversible pulpitis and cold hyperalgesia. J Endod. 2007 Oct;33(10):1167-71. https://doi.org/10.1016/j.joen.2007.06.018
34. Ren K, Dubner R. Interactions between the immune and nervous systems in pain. Nat Med. 2010 Nov;16(11):1267-76. https://doi.org/10.1038/nm.2234
35. Rechenberg DK, Galicia JC, Peters OA. Biological markers for pulpal inflammation: a systematic review. PLoS One. 2016 Nov;11(11):e0167289. https://doi.org/10.1371/journal.pone.0167289
36. Linley JE, Rose K, Ooi L, Gamper N. Understanding inflammatory pain: ion channels contributing to acute and chronic nociception. Pflugers Arch. 2010 Apr;459(5):657-69. https://doi.org/10.1007/s00424-010-0784-6

Evaluation of the expression of nerve fiber markers in healthy and inflamed dental pulp. Braz Oral Res. 2023;37:e020.

Legend

Where is read: *de Arruda JAA, Gomes HS, Sampaio FC, Bruno KF, Estrla C, Mendonça EF, et al.*

Should read: D*e Arruda JAA, Gomes HS, Sampaio FC, Bruno KF, Estrla C, Mendonça EF, et al.*

https://doi.org/10.1590/1807-3107bor-2023.vol37.0020.erratum