

ORIGINAL RESEARCH

The relationship of pulp polyp with the presence and concentration of immunoglobulin E, histamine, interleukin-4 and interleukin-12

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Keywords

allergy, cytokine, histamine, hyperplastic pulpitis, IgE, pulp polyp.

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doi:10.1111/j.1747-4477.2009.00160.x

Abstract

The aim of this study was to evaluate the relationship between pulp polyp formation and immunoglobulin E (IgE), histamine and interleukin-4 (IL-4) as the most important mediators which are involved in allergy. Thirty-two samples including 16 pulp polyps and 16 normal pulps were gathered. After homogenising the pulpal tissue samples, enzyme-linked immunosorbent assay (ELISA) techniques were used to assess the concentration of IgE, histamine, IL-4 and IL-12. The two groups showed statistically significant differences in terms of both the concentration and presence of IgE, histamine and IL-4 ($P < 0.001$); both presence and concentration of IgE, histamine and IL-4 were higher in pulp polyps than in normal pulps. There is not any significant difference between case and control groups regarding IL-12. The results of this study give rise to the possibility of type I hypersensitivity reaction being involved in pulp polyp's pathogenesis.

Introduction

Apart from microorganisms that act as the major cause of dental caries and pulpal disease, pulpal defence against irritants also seems to be involved in the pathogenesis of pulpal disease. The presence of immune cells and various types of immunoglobulin and inflammatory mediators in the inflamed pulp indicates the role of the immune system in the process of pathologic change in the dental pulp (1–4). Although the dental pulp is equipped with cells of immune system, the immune response in the pulp to caries pathogenesis is poorly understood (5). A chronic pulpal inflammation under caries is likely elicited by bacterial antigens that diffuse into the pulp through dentinal tubules (6–8). Immunohistological studies of dental pulps under shallow caries have revealed a lesion that is restricted almost exclusively to T cells, with CD8⁺ T cell predominating (9,10). As the carious lesion enlarges and invades the inflamed dental pulp, CD8⁺ T cells continue to dominate but CD4⁺ T cells, B cells and plasma cells appear in substantial numbers (9,10). It is reasoned that antigens of caries pathogens may preferentially elicit different

immune cell types during caries invasion and pulpal pathology (11–14). In addition, Walsh suggested that mast cells serve a critical role in the development of inflammation in dental pulp (15). Freitas *et al.* in 2007 found mast cells only in pulp polyps but not in the pulps of unerupted, partially erupted without caries, erupted without caries and erupted teeth with shallow dentin caries. They also reported that pulps from carious teeth contained more CD4⁺ and CD8⁺ T cells than from non-carious teeth, and there was also a significant decrease in the number of lymphocytes in pulp polyps (16).

Among the various inflammatory conditions of the pulp, asymptomatic conditions such as pulp polyp have received little attention. The pulp polyp, also known as chronic hyperplastic pulpitis or proliferative pulpitis, is an uncommon and specific type of inflammatory hyperplasia that is associated with a vital tooth and is usually asymptomatic (17). The pulp polyp is the result of both long mechanical irritation and bacterial invasion into the pulp of a tooth that exhibits significant crown destruction. Bacteria (primarily Gram-positive) are found on the surface of the polyp and within the carious lesion (18).

The hyperplastic tissue reaction occurs because the young dental pulp has a rich blood supply and favourable immune response that is more resistant to bacterial infection (17). The present literature indicates the presence of Russel bodies in chronically inflamed pulps (18) and fewer inflammatory cells in pulp polyps with a history of trauma than those resulted from bacterial invasion (19). In one study, it was reported that radicular pulp tissue in the middle and apical third of root canals in pulp polyp is free from inflammatory cells (20). There are a higher number of mast cells in pulp polyps than in normal pulps, and higher numbers of mast cells in the inflamed pulp (16,21). It is well established that mast cells are the only human tissue cells that contain histamine and express very high affinity receptors for immunoglobulin E (IgE). Tissue mast cells are normally coated with IgE (22–26), indicating that type I hypersensitivity or allergy is involved in the pathogenesis of pulpal disease. IgE and mast cells have an essential role in allergy and hypersensitivity reactions. An encounter with an allergen results in the production of IgE, which upon release attaches to mast cell surface receptors, leading to the cell's sensitivity to the allergen. A second encounter with the allergen causes the mast cells to degranulate and release inflammatory mediators, particularly histamine. Type I hypersensitivity and allergy have also been linked with some types of nasal polyp (22–26). On the other hand, it is well-known that interleukin-4 (IL-4) promotes production of IgE (22,27) and stimulation of mast cells (27).

No studies have so far evaluated the possible relationship between pulp polyp formation and allergy. The aim of this study was to assess such a relationship.

Materials and methods

In this case–control study, data were gathered from subject interview, oral examination, radiographs and immunologic assessment. Samples of pulp polyp were taken from patients referring to the Dental School in the years 2006 and 2007. Subjects were excluded if:

1. they had systemic disease that could affect antibody production, such as immunologic deficiencies and malnutrition;
2. they had taken antibiotics, non-steroidal anti-inflammatory drugs and/or H₂ receptor antagonists;
3. they had a history of severe psychological stress (based on the Holmes–Rahe criteria), radiotherapy, immunosuppression or drug abuse. Women who were pregnant, lactating or in their menstrual period were also excluded from the study.

The local Ethics Committee approved the experimental protocol. After obtaining written consent, 32 subjects were divided into two groups: the case group (16 sub-

jects) with a mean age of 14.06 ± 5.13 years, consisting of 50% female and 50% male; and the control group (16 subjects) with a mean age of 15.69 ± 2.91 years, consisting of 56.25% female and 43.75% male. The age and gender of the subjects of the two groups were matched.

In the control group, normal pulp was accessed by mechanically halving the teeth immediately after extraction. In the case group, pulp polyps were removed with a sterile excavator following access to the pulp chamber. The pulps in both groups belonged to permanent teeth and were immediately washed with ice-cooled RPMI-1640, transferred to sterile plastic microtubes and was frozen at -70°C .

The frozen samples were placed on dry ice-cooled petri dishes and were minced into very small pieces (about 1 mm^3) with a surgical scalpel. For each of case and control groups, six small pieces were added to 15 mL of homogenising buffer (0.25 M sucrose, 50 mM Tris/HCl (pH 7.5), 25 mM KCl, 5 mM MgCl₂) at 4°C . The homogenates were centrifuged at 1800 rpm for 10 min to remove tissue fragments. Then, the enzyme-linked immunosorbent assay (ELISA) method was used in order to measure the concentration of IgE (GD09 total IgE kit, Genesis, UK), histamine (RE59221 Histamine ELISA Kit, IBL, Germany), IL-4 (BMS225/2 Human IL-4 ELISA Kit, Bender MedSystems, Austria) and IL-12 (BMS238 Human IL-12 (p70) ELISA Kit, Bender MedSystems, Austria). The operator and the person responsible for recording the results had no prior knowledge of the group to which the samples belonged.

Statistical analysis was undertaken using *t*-tests. Because of the relatively small sample sizes, non-parametric tests (Mann–Whitney *U*-test, chi-squared test and Spearman's correlation coefficient) were also applied to the data.

Results

IgE was found in 100% of pulp polyps and 50% of normal pulps with mean concentration of 0.81 ± 0.5 and 0.21 ± 0.2 IU/mL, respectively (Fig. 1). Histamine was

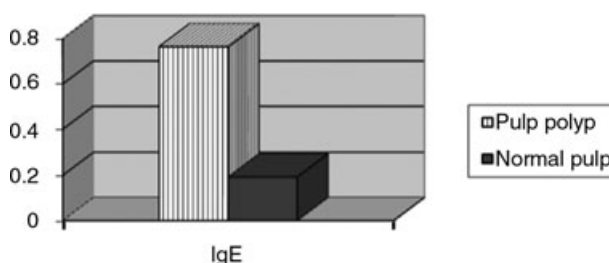


Figure 1 Comparison of the concentration of immunoglobulin E (IgE) (IU/mL) between pulp polyps and normal pulps.

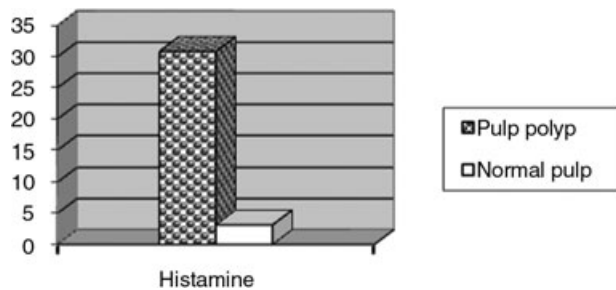


Figure 2 Comparison of concentration of histamine (ng/mL) between pulp polyps and normal pulps.

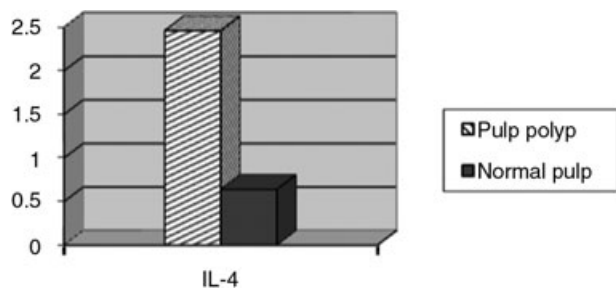


Figure 3 Comparison of concentration of interleukin-4 (IL-4) (ng/mL) between pulp polyps and normal pulps.

found in 100% of pulp polyps and 12.5% of normal pulps with mean concentration of 30.835 ± 34.15 and 3.125 ± 8.73 ng/mL, respectively (Fig. 2). The difference between the two groups was significant in terms of the concentration of histamine ($P < 0.001$). A significant correlation was found between the presence of histamine and pulp polyp formation ($P < 0.001$). A significant positive correlation was also found between the concentration of histamine and pulp polyp formation ($P < 0.001$).

IL-4 was found in 62.5% of pulp polyps and 18.75% of normal pulps with mean concentration of 2.46 ± 2.17 and 0.63 ± 1.40 ng/mL, respectively (Fig. 3). IL-12 was found in 25% of pulp polyps and 12.5% of normal pulps with mean concentration of 0.71 ± 1.84 and 0.20 ± 0.58 ng/mL, respectively (Fig. 4). There was also a significant difference between pulp polyps and normal pulps regarding IL-4 concentration ($P < 0.01$). Although the concentration of IL-12 was higher in pulp polyps than in normal pulps, it was not significant.

Statistical analysis by *t*-test revealed a significant difference in the IgE concentration ($P < 0.001$) between the two groups. There was also a statistically significant correlation between IgE concentration and pulp polyp formation ($P < 0.001$). These differences were also significant by using non-parametric tests.

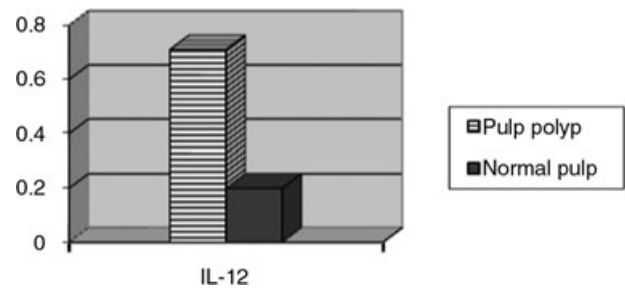


Figure 4 Comparison of concentration of interleukin-12 (IL-12) (ng/mL) between pulp polyps and normal pulps.

Discussion

The pulp polyp, also known as chronic hyperplastic pulpitis or proliferative pulpitis, is the result of both mechanical irritation and bacterial invasion into the pulp of a tooth that exhibits significant crown destruction. The large exposure of pulpal tissue to the oral environment and bacterial invasion results in a chronic inflammatory response that stimulates a hyperplastic tissue reaction, which occurs because the young dental pulp has a rich blood supply and favourable immune response that is more resistant to bacterial infection. There is little information about the exact role of immune factors in pulp polyp.

We found significantly higher concentration of IgE and histamine in pulp polyps compared with normal pulps, and a close correlation between IgE and histamine concentrations.

Zerosi *et al.* in 1999 found a 90% incidence of Russel bodies in chronically inflamed pulps (18); however, it is not clear whether they included pulp polyps in their study. Although they studied factors other than those evaluated in the present study, the findings are in fact similar. This is because they showed active production of immunoglobulin in chronically inflamed pulps, possibly including pulp polyps, and Russel bodies are aggregations of immunoglobulin.

IgE is the most important factor in the stimulation of histamine release from mast cells, and these two factors function in close coordination. Thus, the 100% presence of histamine in pulp polyps is not surprising, considering the high rate of IgE presence in polyps. The predominant mast cell population in the pulp is connective tissue mast cell, which contain greater amounts of histamine than mucosal mast cells. This is consistent with mast cells serving a critical role in the development of inflammation in dental pulps (15). In addition, Shoji *et al.* in 2006 found that lipopolysaccharide (LPS) could be involved in the development of pulpitis or gingivitis, by inducing a marked increase in the histidine decarboxylase (a

histamine forming enzyme) and consequent increase in histamine concentration (12).

In the case of IL-4, as we expected, we found significantly higher levels of IL-4 in pulp polyps than in normal pulps. Although the concentration of IL-12 was higher in pulp polyps, it was not significant. This finding is similar to other studies. For example, Freitas *et al.* in 2007 reported that pulps from carious teeth contained more CD4+ and CD8+ T cells than from non-carious teeth, and there was also a significant decrease in the number of lymphocytes in pulp polyps in comparison with other groups (16). Hahn *et al.* in 2000 suggested that in shallow caries, there is an extraordinary induction of type I cytokines (such as IL-12 and IFN- γ) and preferential activation of CD8+ T cells by *Streptococcus mutans*. In contrast, in deep caries there is no difference in cytokine frequency (11) and Jiang *et al.* in 1999 also reported that *S. mutans* stimulated high levels of IL-12 and IFN- γ . In contrast, *Porphyromonas endodontalis* stimulated production of IL-10 but not IL-12 or IFN- γ (28). It is noteworthy that in the human dental pulp, the predominant mast cell population is connective tissue mast cell which contains greater amounts of histamine than mucosal mast cell and it does not need IL-4 for its differentiation (15). So, the IL-4 in pulp polyps can preferentially induce class switching to IgE rather than mast cell development.

It can be concluded from the above findings that in pulp polyps, continuous irritation by antigens leads to the production of immunoglobulin, particularly IgE. The statistically significant correlation between IgE and histamine in polyps suggests the involvement of type I hypersensitivity or allergy in its pathogenesis. In other words, allergy might be an etiologic factor in cases of pulp polyp, and endodontopathic bacteria and their products, such as LPS, may have some important role in inducing allergy in dental pulps under the extensive caries lesion. Of course, oral pathogens can differ dramatically in their ability to induce different kinds of cytokines. Further study and assessment of other factors involved in allergy such as mast cells and other cytokines is recommended.

Acknowledgements

The authors wish to thank the subjects who participated in the investigation and the Department of Endodontics of the Dental School of the Shaheed Beheshti University of Medical Sciences and Health Services.

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