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Immunocompetent cell level as a diagnostic reference for pulpal pathosis of primary teeth

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ABSTRACT

Objective: The present study was designed to measure changes in the level of immunocompetent cells as healthy pulp becomes inflamed in order to evaluate the use of CD4+/CD8+ and B/CD3+ lymphocyte ratios as a diagnostic reference for pulpal pathosis in primary teeth pulp.

Design: Based on clinical and radiographic examinations, 113 carious and non-carious primary teeth were grouped as healthy teeth, teeth with reversible pulpitis and teeth with irreversible pulpitis. Following dental extraction, pulp samples were collected from all teeth, and 81 of the samples were found to be suitable for flow-cytometric analysis of lymphocyte subset. Kolmogorov–Smirnov's test, One-way ANOVA and Tukey's Post Hoc tests were used for statistical analysis.

Results: Statistical analysis revealed no increases in the mean percentages of T, B and CD4+ lymphocytes in inflamed pulp when compared to healthy pulp. However, both CD8+ and NK cell numbers decreased in line with progressive inflammation. Whereas the CD4+/CD8+ ratios increased in accordance with the severity of pulpitis, B/T ratios remained unaffected.

Conclusions: Immunocompetent cell levels did not change in line with progressive inflammation; therefore, the use of CD4+/CD8+ and B/CD3+ lymphocyte ratios cannot be used as a diagnostic reference for pulpal pathosis in primary teeth.

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1. Introduction

Despite the vast amount of information that has been generated over the past 50 years regarding the reaction of pulp tissue to various irritants such as caries, trauma and microleakage,[1–7] questions still remain regarding how primary teeth pulpal defense mechanisms respond to advancing caries. Recent studies have revealed pulpal immunodefense reactions to be the key factor in determining the ability of pulp tissue to eliminate carious stimuli.[2–17] For this reason, it is important that the pulpal immunosurveillance system is well understood.

Experimental data has shown normal dental pulp to contain a variety of immunocompetent cells, including polymorphonuclear leukocytes, macrophages, CD3+ and B lymphocytes and mast cells. These cells, as well as antibodies to specific bacteria, are the initiators and mediators of immunological response, inducing inflammatory reactions against oral microorganism antigens and participating in the regulation of pulpal pathogenesis.[11,14,18–22]

CD3+ lymphocytes are differentiated into two subsets according to their antigens. Those with CD4+ antigens (helper cells) promote the proliferation and differentiation of immune system cells, whereas those with CD8+ antigens (cytotoxic/

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supressor cells) kill antigen-bearing cells or block the activity of CD4+ lymphocytes and inhibit immune response.[14,18,22–24]

By controlling each other's functions, CD4+ and CD8+ lymphocytes provide the optimal level of immune response. Thus, the stability of CD4+/CD8+ ratio is important for the maintenance of immunological balance.[8,24–26] Jontell et al.[25] demonstrated that CD8+ lymphocytes exist at higher rates than CD4+ lymphocytes in normal dental pulp of permanent teeth. Similar results were obtained by Hahn et al.[8] who asserted that CD8+ cells are the predominant lymphocyte subset in normal pulp, with a CD4+/CD8+ cell ratio of 0.26 in permanent teeth, compared to a 1.14 ratio in permanent teeth with irreversible pulpal pathosis. In contrast to permanent teeth pulp, Angelova et al.[26] found slightly more CD4+ cells than CD8+ cells in healthy primary teeth. However, these results conflict with those obtained from flow cytometric analysis sensitive to cell appointment in the pulp, with, for instance, Mangkornkarn et al.[24] showing more CD4+ cells than CD8+ cells in permanent teeth and Simsek and Duruturk[17] showing more CD8+ cells than CD4+ lymphocytes in primary teeth, regardless of caries status and with no significant alterations in the CD4+/CD8+ ratio with increasing total cell numbers.

Immunohistological studies[12,27] have shown the immunological interaction between T and B lymphocytes to be responsible for the destruction or protection of pulp by or from dental caries. Izumi et al.[12] found the number of B lymphocytes to exceed the number of CD3+ lymphocytes in permanent teeth. Hahn et al.[8] observed a correlation between the numbers of B and CD3+ lymphocytes, and they suggested a B/CD3+ ratio of 1.60 as an index for the immunohistological diagnosis of irreversible pulpal pathosis in permanent teeth.

Since the success of pulp therapy depends on the healing capacity and defense potential of pulp tissue, a correct diagnosis of pulpal status is very important for treatment planning.[2,15,22] However, the lack of correlation between clinical signs and symptoms and primary dental pulp histopathological status makes it difficult to determine what modality offers the best chance for long-term success in the treatment of carious primary teeth.[5,6,17,28–30] Simsek and Duruturk[17] have shown that pulp tissue maintains its healing and defense capacity in the face of advancing carious lesions in primary teeth. In that study, when the CD4+/CD8+ and B/CD3+ values used as indicators for the immunohistological diagnosis of pulpal pathosis in permanent teeth were applied to primary teeth, there was no apparent evidence of irreversible pulpal pathosis in primary pulp. However, this result conflicts with daily clinical observations. In fact, numerous authors have stated that primary pulp tissue, especially in teeth with deep carious lesions, is unable to defend itself and that necrosis can occur even in unexposed pulp.[1,31–36] Given these findings, it is possible to suggest that the CD4+/CD8+ and B/CD3+ values used as indicators of pathosis in permanent teeth pulp may not be the same for primary teeth pulp. For this reason, CD4+/CD8+ and B/CD3+ lymphocyte ratios must first be determined as an index for the immunohistological diagnosis of irreversible pulpal pathosis in primary teeth. Once this has been accomplished, CD4+/CD8+ and B/CD3+ values must be estimated for teeth with varying depths of caries (a matter for another study). By checking these values against the index values, it will be possible to predict the depth of the lesion at which irreversible pulp inflammation has occurred, which can in turn lead to a correct diagnosis of pulp status.

The present study was designed to measure changes in the level of immunocompetent cells as healthy pulp becomes inflamed in order to evaluate the use of CD4+/CD8+ and B/CD3+ lymphocyte ratios as a diagnostic reference for pulpal pathosis in primary teeth pulp.

2. Materials and methods

2.1. *Experimental material*

A total of 113 primary teeth were obtained from 58 healthy and cooperated children aged 6–13 years requiring dental extraction for orthodontic treatment or other indications at the Ankara University Faculty of Dentistry's Paediatric Clinic in Ankara, Turkey. The research protocol was reviewed and approved by the Faculty of Dentistry Ethics Committee, and informed written consent was obtained from the parents of all children who participated in the study.

Both carious and non-carious maxillary and mandibular incisors, canines and molars were included in the study. Teeth with periapical or interradicular pathosis, physiological or pathological root resorption, calcific degeneration of pulp tissue, internal resorption, sensitivity to percussion or palpation, physiological or pathological mobility, oedema or sinus tract were excluded from the study. Based on clinical and radiographic examinations, study groups were comprised according to criteria suggested by Bowles et al.[37] and Abbott and Yu[38], as follows:

Group A – Healthy teeth

1. No caries present.
2. No radiographic evidence of periapical pathosis.
3. No spontaneous pain.
4. No pain percussion or chewing.

Group B – Teeth with reversible pulpitis

1. Clinical and radiographic evidence of deep carious lesion.
2. Transient response to cold stimuli applied by a cotton pellet saturated with the ethyl chloride (Chloraethyl, IGS AEROSOLS GMBH-Im Hemmet 1-D-79664 Wehr/Baden-GERMANY).
3. No spontaneous pain.
4. 4-No radiographic evidence of periapical pathosis.

Group C – Teeth with irreversible pulpitis

1. Clinical and radiographic evidence of deep carious lesion.
2. History of spontaneous pain, which may wake the patient at night.
3. A tooth responsive to cold with a positive lingering response.
4. No radiographic evidence of periapical pathosis.

Teeth were extracted under general anaesthesia or using an anaesthetic solution that did not contain a vasoconstrictor (3% Isocaine, Novocol Pharmaceutical, Canada). Teeth were

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