LEVELS OF TNF-α IN ASYMPTOMATIC APICAL PERIODONTITIS

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ABSTRACT

Introduction

Asymptomatic apical periodontitis (AAP) is a chronic inflammatory process mediated by a milieu of pro-inflammatory cytokines. TNF-α is a key mediator and a potent inducer of bone resorption and connective tissue degradation. The aim of the study was to examine TNF-α production by peripheral blood mononuclear cells (PBMCs) from patients with AAP and to explore its correlation to the lesions' characteristics.

Materials and methods

Patients (n=20) diagnosed with AAP were included in the study. The lesions' characteristics were assessed with limited-volume cone beam-computed tomography (limited FOV CBCT). PBMCs were isolated from peripheral blood samples obtained from each patient. The cells were cultivated in RPMI 1640 medium and stimulated with *E.coli* LPS. The supernatant was collected at the 24th and 48th-hour time point. TNF-α levels were detected by ELISA.

Results

TNF-α concentrations decreased significantly at the 48-h time point compared to the 24-hour time point ($p<0.01$). Unstimulated PBMCs produced higher levels of TNF-α compared to stimulated ones. No statistically significant correlation was found between the lesions' variables and IL-1β levels.

Conclusions

We suggest that TNF-α secretion is more impactful to the inflammatory process in the first 24 hours.

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KEYWORDS: *TNF-α, Asymptomatic apical periodontitis, PBMCs*

Introduction

Asymptomatic apical periodontitis (AAP) is one of the most common chronic inflammatory diseases in the field of dental medicine. It represents a chronic immune response to a persistent microbial infection of the root canal system that affects the apical periodontal ligament and the adjacent alveolar bone and cement and results in peri-radicular tissue destruction [1]. The alveolar bone resorption results in the formation of an osteolytic apical lesion.

In the field of periodontology, recent research has indicated that the major determinant of chronic marginal periodontitis' dynamics is the nature of the immunoinflammatory response [2]–[4]. It is paradoxical that the initially protective immune reaction is responsible for the majority of tissue damage that leads to the clinical manifestations of the disease [2] This underlines the leading role of the patient's immune reactivity for the progression of the

disease, and the solely initiating and perpetuating role of the biofilm. The evidence is based on research on secreted mediator levels by peripheral blood mononuclear cells (PBMCs) isolated from patients with chronic marginal periodontitis and their association with the clinical parameters of the disease [5]–[9]. Whether the same applies for the development of chronic asymptomatic apical periodontitis, however, remains largely unexplored.

One of the investigated mediators is TNF-α , a cytokine released as a result from the interaction between the endodontic biofilm and the host defensive cells. TNF-α is a monocyte-derived protein with wide-ranging pro- and inflammatory effects on various cell populations [10]. It is a mediator present in periapical lesions, and its role as a determinant of disease progression has been previously proven in chronic marginal periodontitis [11] [12]. In AAP, TNF-α is a key pro-inflammatory cytokine that upregulates the expression of other inflammatory mediators and potentiates the inflammatory response. It activates leucocytes and endothelial cells, stimulates the production of chemokines and induces bone resorption through the upregulation of prostaglandins and MMPs [13]. Inappropriate expression or overproduction of TNF-α can lead to a variety of pathological conditions [14]. The aim of the study was to examine TNF-α production by PBMCs from patients with AAP and to explore its correlation to the apical lesions' characteristics.

Materials and methods

Patient Selection and Enrollment

Twenty patients with a single asymptomatic periapical lesion, intact dentition and clinically healthy marginal periodontium were enrolled in the study. Exclusion criteria for all subjects included any systemic diseases and disorders, as well as intake of medication in the last three months prior to the beginning of the research. The study was approved by the Ethics Committee of Medical University – Plovdiv and conducted according to the criteria set by the declaration of Helsinki. Informed consent was acquired from all participants.

Lesion Assessment

AAP diagnosis was based on clinical and radiographic findings. The assessment of the apical lesion's characteristics was carried out with limited-volume cone beam-computed tomography (limited FOV CBCT). Lesion size was determined by measuring its diameter and calculating its volume.

Laboratory Methods

PBMCs were isolated from peripheral blood samples obtained from each patient. Deposited heparinized blood was used as the source of PBMCs. The cells were isolated within an hour by Ficoll gradient, according to the manufacturer's instructions. The cells were suspended in RPMI 1640 medium divided into two fractions. One fraction was stimulated with E.coli LPS, and the other was left unstimulated to serve as a negative control. The supernatant from both fractions was collected at the 24th and 48th-hour time point for cytokine detection. TNF-α concentrations were determined by commercially available ELISA kit (BioLegend, San Diego, CA) per manufacturer's instructions with a minimum detection limit of 3.5 pg/mL. SPSS software (version 17; SPSS Inc., Chicago, IL) was used for data processing and statistical analysis. The means and standard deviations were calculated for all the parameters and Wilcoxon's signed ranks test was performed. Fold change and delta difference were determined by dividing the cytokine concentration of stimulated PBMCs for every patient by the corresponding unstimulated PBMC cytokine value. Spearman's rank correlation coefficient was used to assess correlations between TNF-α levels and the lesions' diameter and volume.

Results

TNF-α concentrations decreased significantly at the 48-hour time point compared to the 24-hour time point ($z=-2,667$, $p<0.01$). Qualitative differences in the cytokine response pattern between stimulated with LPS and unstimulated PBMCs were observed. Untreated PBMCs produced significantly higher levels of TNF-α compared to stimulated ones at both measurement time points ($z=-2,100$, $p<0.05$). No statistically significant correlation was found between the lesions' variables and TNF-α levels.

Discussion

To investigate the possible destructive role of the immune response in the pathogenesis of AAP, we enrolled patients diagnosed with a single chronic apical lesion. Following clinical data collection, we isolated PBMCs from the peripheral blood of each subject in order to examine the response to stimulation with standardized extracts of bacterial toxins. While the local immune response is not solely determined by PBMCs, they represent the systemic immune status and differentiate into macrophages, which are the major contributors to tissue breakdown in periodontal diseases.

The egress of bacteria and their by-products from the apical foramen to the periapical tissues triggers an immune response mediated by a large number of pro-inflammatory cytokines [15]. The prolonged release of inflammatory mediators triggers the pathophysiological processes of bone destruction and leads to the formation of an apical lesion. TNF-α is believed to play a fundamental role in the pathogenesis of AAP and the consequent bone resorption [16]. We examined the TNF-α production by PBMCs in response to LPS stimulation. Our special interest was also to determine the association between the cytokine response and the destruction extent of the disease.

We observed that TNF-α levels were higher at the 24th-hour time point. Therefore, we suggest that TNF-α secretion is more impactful to the inflammatory process in the first 24 hours following pathogen exposure. However, no correlation between TNF-α concentrations and lesion size was established. A direct association between TNF-α levels and the bone destruction extent cannot be assumed. We suggest that this is attributable to the fact that TNF-α is not the only determinant of bone resorption, as the process is orchestrated by a milieu of pro-inflammatory cytokines. However, further studies should follow up on the results described in our study in order to characterize the connection between the systemic factors and AAP. New findings in this area can shed light on the clinical and paraclinical characteristics of the disease and expand our understanding of its dynamics. Elucidation of the intricate pathogenetic mechanisms of AAP is the foundation for the development of advanced diagnostic techniques, the improvement of therapeutic outcomes and the optimization for the long-term prognosis of the disease.

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