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Association study between salivary levels of interferon (IFN)-gamma, interleukin (IL)-17, IL-21, and IL-22 with chronic periodontitis

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ABSTRACT

Objective: To investigate if the salivary levels of IL-17, IL-21, IL-22, and its ratio regarding salivary IFN-γ may be linked with the periodontal clinical status.
Design: One hundred and five chronic periodontitis (CP) subjects and 44 healthy controls (HC) were recruited. Periodontal status was assessed based on full-mouth clinical periodontal measurements. Cytokine salivary levels were analyzed by ELISA. The association between the analytes with CP was analyzed using a binary logistic regression model.
Results: A statistically significant increase in salivary levels of IFN-γ and IFN-γ/IL-22 ratio in CP group could be detected, but there was no significant domination of any Th17 cytokine that could be of predictive value for health/disease status. Univariate and binary logistic regression analyses revealed a strong and independent association of IFN-γ salivary levels and IFN-γ/IL-22 ratio with disease status. An interaction effect of ageing on IFN-γ levels also could be noted.
Conclusion: While salivary levels of IFN-γ and IFN-γ/IL-22 ratio may act as strong/independent indicators of the amount and extent of periodontal breakdown, the low detection frequency of Th17 cytokines in saliva samples make these determinations useless for the detection of disease presence and/or its severity.

1. Introduction

Periodontal diseases are progressive and destructive inflammatory conditions of the tooth-supporting tissues of multifactorial nature with pathogenesis related to several risk factors, including bacteria, host responses, and genetics. Although it has been recognized that chronic periodontitis (CP) is initiated and maintained in the first line by a complex polymicrobial infection,[1] it is now thoroughly accepted that the innate susceptibility of the patient is the critical factor that will determine the destructive character of the disease. In this way, bacterial virulence factors either result directly in degradation of host tissues or induce the release of inflammatory mediators and chemokines that lead to tissue destruction.[2]

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Among these biologic mediators, interferon (IFN)-γ, a pleiotropic cytokine, has demonstrated to possess potent proinflammatory actions in innate and adaptive immune responses not only by inducing the production of cytokines and chemokines,[3,4] but also by upregulating the expression of various membrane proteins including class I and II major histocompatibility complex (MHC) antigens, Fc receptors, leukocyte adhesion molecules, and B7 family antigens. Furthermore, IFN-γ is a potent activator of macrophage effector functions. It directs the synthesis, class switching, and secretion of immunoglobulins by B cells.[5,6]

The activation of innate immunity almost synchronously leads to the activation of cell-mediated immunity.[7] In this context, cytokines produced after the initial activation lead to the differentiation of naive CD4+ T cells towards one of the three functional subpopulations known today as T-helper (Th)1, Th2, and Th17 cells, based on the cytokine profiles they secrete. Since the initial description of Th1 and Th2 subpopulations,[8] it has been attempted to explain the immunopathogenesis of periodontal diseases in one or other response profile, which has generated enormous controversy among researchers.[2] Only until 2005, it was recognized the subset Th17 as a lineage of distinct CD4+ T cells,[9] responsible for the production of the cytokines interleukin (IL)-17A, IL-17F, IL-21 and IL-22,[10] which not only have a pro-inflammatory character at mucosal surfaces in response to extracellular pathogens[11,12] but also have been associated with autoimmunity.[13] At this point, it is possible to argue that Th1, Th2, and Th17 subsets are potentially destructive from the tissue damage viewpoint.[4] Nevertheless, it has been stated that IFN-γ influences Th cell phenotype development by inhibiting the differentiation of Th2/Th17 cell lineage and of the stimulation of Th1 development.[5,6,9]

Although several studies performed in gingival crevicular fluid (GCF) and gingival tissue samples have shown that the expression of both IFN-γ and/or Th17 cytokines might play a role in the etiopathogenesis of CP,[14–21] there is only limited information concerning the levels of these immunoregulatory factors in saliva samples and its relationship with the clinical manifestations of disease.[22–24] As saliva has gained significant recognition as a biological sample for the detection of biochemical and cellular factors that may reflect the biological changes related to tissue damage observed in CP,[24–28] this study aimed to investigate if the salivary levels of IL-17, IL-21, IL-22, and its ratio regarding IFN-γ salivary levels may be linked with the periodontal clinical status in a Colombian population.

2. Materials and methods

2.1. *Study population and inclusion/exclusion criteria*

This descriptive, cross-sectional study was conducted at the Faculty of Dentistry, University of Antioquia in Medellín (Colombia) from January 2011 to December 2013. The study conformed to the ethical guidelines of the Helsinki Declaration and was evaluated and approved by the Institutional Ethics Committee for Human Studies. The sample size was calculated using a Sample Size Calculator (Raosoft® Inc., Seattle, WA, USA) on the basis of a previous study regarding the association of salivary levels of various cytokines with periodontal disease.[29] It was increased by 20% to safeguard the estimates at an optimal level of precision (5%) against the possible effect of size reduction due to exclusions and dropouts. Thus, the theoretical sample size for clinical screening was set to 141 subjects (distributed between two groups) to determine significant differences in outcomes at the 95% confidence level, with an alpha value = 0.05 and 80% power. However, every effort was made to recruit the maximum number of subjects, so that the study sample included a total of 149 subjects from the population of individuals that sought treatment and/or consults at the Graduate Periodontics Clinics.

The purpose was fully explained, and appropriate informed consent was obtained from all subjects prior to their enrolment into the study. Participants were privately interviewed to obtain demographic and medical information and underwent a clinical screening for presence of oral pathology and a periodontal examination. The information that was gathered included the subject's gender, age, medications, systemic health, and smoking habit. A subject was considered as non-smoker if he or she had never smoked, or had stopped smoking more than 5 years previous to the date of examination.[30] All clinical periodontal measurements were performed by two trained and calibrated observers. The clinical parameters recorded, including probing depth (PD) and clinical attachment level (CAL), were measured at six surfaces of all teeth (midbuccally, midlingually, and proximally both buccally and lingually) to the nearest mm, using a manual periodontal probe (PCP UNC 15, Hu-Friedy, Chicago, IL, USA). Following previously defined criteria,[31] these measurements were used to calculate the extent and severity of periodontitis based on the percentage of tooth sites having PD $\geq$4 mm along with CAL $\geq$2 mm (extent) and the average value of attachment loss of the diseased sites (severity). All calculations were based on data derived from the full-mouth examination.

Subjects for this study comprised 105 untreated chronic periodontitis (CP) subjects and 44 healthy controls (HC). These patients were classified as follows: CP, subjects having a minimum of 20 remaining teeth, with periodontal disease as evidenced by at least four tooth sites with PD $\geq$4 mm and CAL $\geq$2 mm,[32] and radiographic evidence of bone loss >2 mm from the cemento-enamel junction.[25] HC, subjects with no evidence of pocket depth >3 mm and no clinical gingival inflammation (no more than 10% of sites with bleeding on probing and absence of gingival redness/oedema), but could have attachment loss or gingival recession due to mechanical trauma. Exclusion criteria were patients who would not give informed consent; diseases of the oral hard or soft tissues, except dental caries and chronic periodontitis; pregnancy and lactation; ongoing orthodontic therapy; any systemic condition that could affect the host's periodontal status and bone metabolism (e.g. osteoporosis, gastrointestinal diseases related to nutrition and mineral metabolism, endocrine diseases, immunological disorders and connective tissue diseases); or that would require pre-medication for monitoring or treatment procedures (e.g. heart conditions, joint replacements, hormonal or bisphosphonate antiresorptive therapies and chronic