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Salivary levels of inflammatory cytokines and their association to periodontal disease in systemic lupus erythematosus patients. A case-control study

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ABSTRACT

Both Systemic Lupus Erythematosus (SLE) and periodontal disease (PD) present a similar immunological profile mainly characterized by altered cytokine levels. In this study we sought to investigate the salivary levels of inflammatory cytokines and their association with PD in SLE patients. 60 patients with SLE and 54 systemically healthy individuals underwent a full periodontal clinical examination. They were then grouped according to their periodontal status. Stimulated saliva was collected in order to evaluate the salivary levels of interferon (IFN-γ), Interleukin (IL)-10, IL-17, IL-1β, and IL-4. Systemically healthy individuals with periodontitis (group P) presented higher levels of cytokines when compared to systemically healthy individuals, with no periodontal disease (group S) ($p < 0.05$). Additionally, in the P group, patients presented similar levels of cytokines to those of the patients with SLE, regardless of the presence of PD ($p > 0.05$), for most of the analyzed cytokines. There was a positive correlation in SLE patients, including IL-1β and all periodontal clinical parameters ($p < 0.05$), and between IL-4 and gingival bleeding index and the presence of biofilm ($p < 0.05$). Thus, our results confirmed, that patients with PD showed higher salivary levels of cytokines and, in SLE patients, the increased levels of salivary cytokines were observed even in the absence of periodontitis. IL-1β and IL-4 salivary levels were also positively correlated with periodontal status indicating their potential as markers of the amount and extent of periodontal damage in patients with SLE.

1. Introduction

Periodontal disease (PD) is an inflammatory process resulting from imbalance in the interaction between microorganisms of the biofilm and components of the immune response in susceptible individuals. PD can lead to irreversible damage of periodontal support tissues [1]. PD is highly prevalent among different populations worldwide, possessing an important impact factor on oral health programs [2]. The main pathogenic bacteria in PD are anaerobic gram-negative species, that express a number of potential virulence factors thus causing the imbalance in the production of various molecules and inflammatory factors, which are important determinants in the development of the disease [3]. Common signs of PD include bleeding gums, alveolar bone resorption, periodontal pocket formation, halitosis, tooth mobility, and spontaneous tooth loss in advanced cases [4].

Systemic lupus erythematosus (SLE) on the other hand is a chronic systemic autoimmune disease, affecting cellular metabolism of connective tissue, presenting alternate periods of remission and exacerbation [5,6]. SLE presents diversified manifestations and progression which may affect multiple organs and cause severe complications [7,8]. SLE is most common in women and its onset is favored by genetic and environmental factors including ultraviolet radiation and certain drugs [9]. Its worldwide prevalence is quite variable, being estimated at 20–150/100,000 and with an

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increasing trend in the number of cases. In the United States it has a higher prevalence and greater impairment of vital organs among the population of African descent, Hispanic and Asian [10]. It is regarded worldwide as a rare disease. Notwithstanding, SLE has a high incidence in the Brazilian Northeast, with an annual incidence twice higher than that of the UK [11]. In the US from 2002 to 2004, the incidence was approximately 5.6/100,000. These figures when adjusted, point out an incidence five times higher for women than for men [12]. The high incidence of SLE in northeastern Brazil, is still an intriguing finding calling for an evaluation of the impact of SLE in other Brazilian geographical regions, in order to elucidate the factors leading to heightened levels of the disease in that region [11].

A number of studies have demonstrated a possible association between chronic periodontitis and systemic rheumatic diseases [13,14]. Studies on the association between periodontitis and SLE are limited, with some studies showing a high prevalence of PD in patients with SLE [15–17], a greater presence of dental biofilm and gingival bleeding [18]. A single study reported that patients with active SLE had severe periodontal loss, with a higher frequency of tooth loss [19]. Fabbri et al. [16] observed that the treatment of PD has a beneficial effect in controlling disease activity in SLE patients under immunosuppressive therapy, suggesting that PD may be a modifiable risk factor for SLE [16].

Although PD and SLE have different etiologic factors, the presence of similar mechanisms of immune response, leading to tissue damage, either with the destruction of periodontal tissue supporting in PD, or by deposition of macrocomplexes and destruction of connective tissues in SLE, may account for the possible association between these diseases [20]. Inflammation and both the innate and adaptive immune systems lead the underlying pathogenic process in both diseases entailing a network of protective and destructive reactions mainly mediated by cytokines [21]. Cytokine profile in peripheral tissue is of high importance since it outlines local mechanisms that influence the overall health state [22]. Therefore, analysis of proteins or biomarkers by the means of immunological or biochemical methods provide significant evidence of health or disease related to various disorders [21].

Salivary cytokine profile has previously been reported in periodontitis patients [22], and has gradually been identified and suggested as a biomarker for various diseases such as cancer, cardiovascular diseases and autoimmune diseases [22–24]. Saliva has gained significant recognition, as a bodily substance used to monitor biochemical and cellular factors that may reflect biological changes associated with tissue damage. The objective of this study was to evaluate the levels of salivary inflammatory cytokines and their association with PD in patients with SLE in Northeastern Brazil.

2. Methods

2.1. Study design and groups

This case-control study was approved by the Research Ethics Committee of the University Hospital of the Federal University of Maranhão (n° 460.888). The participants were informed about the study objectives and signed the "Informed and Free Consent Form", after fully understanding its contents, which briefly described the study.

All patients, aged between 18 and 60 years, were selected in Sao Luis; a city located in northeastern Brazil. Exclusion criteria were as follows: smokers or former smokers who had ceased smoking in a period of less than 10 years, dental braces users who underwent periodontal treatment in the last six months, clinically evident infectious diseases (except for PD), presence of nodes and/or edema in the region of salivary glands (assessed by visual examination and digital palpation), pregnant women, nursing mothers and/or individuals who had made use of antibiotics in the last 6 months. The participants were divided in four groups: 30 subjects with SLE and without periodontitis (LS Group); 30 subjects with SLE and chronic periodontitis (LP Group); 27 systemically healthy and without periodontitis subjects (S group); and 27 systemically healthy individuals with chronic periodontitis (P Group).

2.2. Oral clinical evaluation

The evaluation and registration of medical and dental history of the patient were conducted through individual questionnaire to identify risk factors for systemic and oral health of patients.

The periodontal examination was performed by a single examiner, previously trained under artificial light and included the following parameters: probing depth (PD), clinical attachment level (CAL), visible plaque index (VPI), gingival bleeding index (GBI). A dental mirror and a Williams probe with a millimetric scale (Trinity, Sao Paulo, Brazil) were used in the clinical evaluation. The intra-examiner correlation coefficient was calculated for the PD (0.84) and CAL (0.81) by Kappa index.

The periodontal examination was performed in all teeth present, through standardization at six sites (distobuccal, med-buccal, mesiobuccal, distolingual, med-lingual and mesiolingual) [25]. The following parameters were recorded: probing depth (PD), distance of the gingival margin to the apical point of gingival sulcus fund or pocket (in mm); clinical attachment level (CAL), distance of cement-enamel junction to the bottom of the gingival sulcus or pocket (in mm); visible plaque index (VPI) was recorded for each individual as the percentage of tooth surfaces with biofilm visible to the oral examiner. Gingival bleeding index (GBI) was also recorded. The GBI and VPI evaluated the presence or absence of inflammation or plate binomial pattern (dichotomous), as follows: gingival margin bleeding and visible plate - score 1 and no bleeding and no visible plaque - score 0 [26]. Patients who presented CAL $\geqslant$ 6 mm in 2 teeth and 1 or more sites with PD $\geqslant$ 5 mm were considered for diagnosis of chronic periodontitis [27].

2.3. Analysis of the saliva

The following cytokine analysis were performed: IFN-γ, IL-10, IL-17, IL-1β, IL-4 and IL-6, through saliva collected by stimulated saliva collection method [28], with the addition of cocktail protease inhibitor (Sigma-Aldrich, USA) and stored at $-80\,^{\circ}C$ until analysis. The concentrations of the above mentioned cytokines were simultaneously measured by the automatic analyzer MAGPIX® System (EMD Millipore, USA) using the 90K-CCYTO Kit, Milliplex MAP (Millipore, Massachusetts, USA) following the manufacturer's instructions.

2.4. Statistical analysis

Data were analyzed using SPSS software (version 17.0). Descriptive statistical analysis was performed using the frequencies, mean and standard deviation. For numeric variables, initially, the normal distribution was analyzed using the Shapiro-Walk test. After this procedure, the comparative analysis of inflammatory markers between the groups was performed using the Kruskal-Wallis test followed by Dunn's multiple-comparison tests. Correlation analysis between salivary levels of inflammatory markers and periodontal parameters in patients groups with SLE was processed by calculating the Pearson's correlation coefficient. The significance level adopted was 5% ($p < 0.05$).