Journal of Oral Biology and Craniofacial Research 6 (2016) S24–S28

Contents lists available at ScienceDirect

Journal of Oral Biology and Craniofacial Research

journal homepage: www.elsevier.com/locate/jobcr

Original Article

sIgA, peroxidase and collagenase in saliva of smokers aggressive periodontal patients

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ARTICLE INFO

Article history:
Received 1 December 2015
Accepted 29 May 2016
Available online 3 September 2016

Keywords:
Saliva
Smokers
Aggressive periodontitis

ABSTRACT

Background: Tobacco smoking is strongly associated with destructive periodontal disease, alveolar bone loss and poor response to periodontal therapy. The aim of this study was to investigate the effect of smoking on antimicrobials and destructive proteins in the saliva of patients with generalized aggressive periodontal disease.
Material and methods: The study group consisted of 55 adult subjects. They were classified according to their clinical diagnoses either as having generalized aggressive periodontitis ($n = 27$; 13 smokers and 14 non-smokers) or as periodontitis-free control group ($n = 28$; 14 smokers and 14 non-smokers). Total proteins, sIgA, peroxidase and collagenase were quantified in whole saliva. Data were analyzed using one-way ANOVA and Tukey tests.
Results: Statistical differences were found between smokers with periodontal disease and healthy control subjects for sIgA, and between non-smokers with periodontal disease and healthy control subjects for peroxidase. Collagenase activity was higher in smokers and non-smokers with periodontal disease, and tobacco use was found to increase collagenase activity in healthy subjects.
Conclusion: Whole saliva from all patients with periodontal disease had higher sIgA and lower peroxidase content. Smokers with periodontitis had higher sIgA than smokers without periodontitis. Smokers with and without periodontitis had higher collagenase activity than non-smokers (with and without periodontitis).

1. Introduction

Periodontal diseases are chronic inflammatory entities characterized by infiltration of leukocytes, loss of connective tissue and alveolar bone resorption.[1] Aggressive periodontitis (AgP) was redefined as a complex disease exhibiting microbial alteration and cellular dysfunction, which differs from chronic periodontal disease in the underlying molecular mechanisms of its pathogenesis.[2] It comprises a heterogeneous group of periodontal diseases that affect adolescents and young adults. It is characterized by very rapid loss of periodontal tissue in otherwise clinically healthy subjects, and can be classified into localized (LAgP) and generalized forms (GAgP). Previous studies have investigated some factors that may increase host susceptibility to tissue destruction, including genetic factors, functional defects of polymorphonuclear (PMN) leukocytes and monocytes, and environmental risk factors. Cigarette smoking may be an important contributor to the development of periodontal disease. Evidence suggests that periodontitis is more prevalent in smokers than in non-smokers. Tobacco smoking is strongly associated with destructive periodontal disease, alveolar bone loss and poor response to periodontal therapy.[3] This suggests that smoking might interfere with the host's immune response. Tobacco metabolites suppress neutrophil function and inhibit the immune response. Nicotine has adverse effects on the role of PMN, altering the function of chemotaxis and phagocytosis and interfering with the production of immunoglobulins (Igs). Cigarette smoking can alter T-cell immune regulation and B-cell differentiation, generating a decrease in the production of Igs, which protect the oral mucosa against periodontal pathogenic bacteria.[4]

PMNs appear to play a pivotal role in aggressive periodontitis. Neutrophil granules are responsible for the neutrophil function and can be divided into three main types, according to their azurophil granule constituents. Myeloperoxidase is the most

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http://dx.doi.org/10.1016/j.jobcr.2016.05.003
2212-4268/

effective microbial and cytotoxic mechanism of leucocytes. Oral peroxidase is composed of two enzymes: salivary peroxidase, which contributes 80% of oral peroxidase, and myeloperoxidase which contributes the remaining 20%.[5]

Damage to periodontal tissue is usually detected by means of periodontal probing, which shows loss of attachment of the tooth, or by radiographs that detect alveolar bone loss. These methods also evaluate the damage caused by previous destruction episodes, resulting in a retrospective diagnosis. Increasing attention is being given to saliva testing due to the need for a simple, non-invasive method for assessing drug intoxicated patients. Collecting saliva is easy, involves neither stress nor risk of injuries from needles, and patients can collect it themselves after instruction. Saliva contains a wide array of components that are very sensitive to toxic substances, and reflects a real-time level of biomarkers.[6]

Some inflammatory mediators and other molecules originated from tissue destruction have been detected in gingivo-crevicular fluid (GCF) and saliva of patients with chronic periodontitis (ChP) or adult periodontitis.[7,8] Results of our previous studies have shown significant statistical differences in certain GCF markers and whole saliva between patients with ChP and periodontally healthy subjects[9,10] and between patients with AgP and periodontally healthy individuals.[11]

The aim of this study was to investigate the effect of smoking on total salivary sIgA, peroxidase and collagenase in patients with generalized aggressive periodontal disease and to investigate the relationships with their clinical parameters.

2. Material and methods

2.1. Clinical parameters

Clinical considerations for patients with periodontitis have been published previously,[9] taking into consideration an adaptation of Lindhe.[12]

Clinical parameters were assessed at six sites on each tooth (mesio-buccal, medio-buccal, disto-buccal, mesio-lingual, medio-lingual, disto-lingual) using a manual periodontal probe (Hu-Friedy, NC, USA). The following parameters were included: gingival index (GI),[13] plaque index (PI),[14] bleeding on probing (BOP) up to 15 s after gentle testing, probing depth (PD) (distance between the gingival margin and the bottom of the sulcus/pocket) and clinical attachment level (CAL) (distance between cement-enamel junction and the bottom of the sulcus/pocket). Bone resorption in GAgP was established on the basis of clinical and radiographic criteria. Periapical radiographs were taken using a standardized long-cone paralleling technique. Patients with GAgP included in this study had moderate (M) to advanced (A) forms of the disease and exhibited at least one site with the clinical features M: GI > 1, PI $> 20\%$, BOP, and PD and CAL between 5 and 6 mm; A: GI > 1, PI $> 20\%$, BOP, and PD and CAL ≥ 7 mm; C subjects: GI < 1, PI $< 20\%$, no BOP, PD ≤ 3 mm and no CAL.

2.2. Study population

The study group consisted of 55 adults. They were classified according to their clinical diagnoses as having generalized aggressive periodontitis (GAgP) ($n = 27$) aged 26.9 ± 4.3 yrs, of whom 13 were smokers (sGAgP) (6 females, and 7 males) and 14 were non-smokers (nsGAgP) (6 females and 8 males). The control group (C) consisted of 28 periodontitis-free adult subjects aged 25.8 ± 2.9 yrs, of whom 14 were smokers (sC) (7 females and 7 males) and 14 were non-smokers (nsC) (6 females and 8 males). All subjects attended the School of Dentistry at the National University of Tucumán for periodontal consultation. Written informed consent was obtained from all patients prior to participation.

Inclusion criteria for all subjects were a minimum of 20 natural teeth excluding third molars GI > 1, PI > 1, BOP, PD and CAL ≥ 4.5 mm for patients and GI < 1, PI < 1, no BOP, PD and CAL < 3 mm for the C group.

Exclusion criteria for all subjects diagnosed with GAgP included systemic diseases, periodontal therapy prior to saliva collection, and use of antibiotics, steroidal or non-steroidal anti-inflammatory agents in the 6 months prior to the study.

Individuals who had smoked more than ten cigarettes per day for at least 5 years were considered smokers.[15]

2.3. Saliva collection

Patients were instructed not to eat or drink for 2 h prior to sample collection. Unstimulated whole saliva was collected at the initial visit, before periodontal treatment, between 8 and 10 a.m. for 10 min. The smoking volunteers were asked not to smoke for 1 h prior to the experiment. While in a sitting position, the participants were asked to swallow saliva, then remain motionless and allow the saliva to drain passively for 10 min over the lower lip into a sterile plastic vial on ice. Saliva accumulated in the antero-vestibular and sublingual regions of the mouth was collected with a saliva ejector, avoiding contact with the mucosa, and placed in a test tube on ice. Whole saliva was centrifuged at 10,000 rpm for 10 min at 4 °C and immediately frozen at −20 °C until chemical determinations were performed.

2.4. Chemical determinations

20 μl of saliva were used to quantify total proteins following Lowry (1951).[16] sIgA was determined by the radial immune diffusion method by placing 5 μl of total saliva twice (Diffu Platte Lab, Argentina). Peroxidase was quantified following Mansson-Rahemtulla et al. (1986).[17] 50 μM 5,5-dithionitrobenzoic acid in 100 mM phosphate buffer pH 5.6 were placed in a 2 ml spectrophotometer cuvette and 2-mercaptoethanol was added in order to obtain an absorbance between 0.8 and 1.0 at 412 nm. Then 100 μl of 90 mM potassium sulfocianide, 100 μl of centrifuged total saliva and 100 μl of 2 mM H_2O_2 were mixed. Readings were performed at 10 s and 20 s. Collagenases were analyzed by zymography using the method described by Ingman et al. (1994).[18] 10 μl salivary samples were loaded in each lane of a 12% SDS-PAGE containing 1 mg/ml gelatin as substrate. Collagenase activity was identified on the gel as light bands on a dark background. SDS-PAGE and zymographies were performed with at least 10 individual samples.

2.5. Statistical analysis

Data were analyzed by the SPSS system (11.0). Differences among groups were studied using one-way ANOVA. When differences were significant, the Tukey test was applied. Variables of the subjects were correlated by means of Spearman rank correlation analysis.

3. Results

Total proteins showed no significant difference ($p > 0.05$) between GAgP and C groups (Fig. 1a). Considering smoking, total proteins were lower in sC than in nsC, although the difference was not significant ($p > 0.05$) (Fig. 1b).

Peroxidase was statistically lower in GAgP than in C ($p < 0.05$) (Fig. 1c). Considering smoking, we found statistical differences ($p < 0.05$) for peroxidase between nsGAgP and nsC but not between C and GAgP smoking and non-smoking subgroups (Fig. 1d).