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Subgingival microbial profiles of generalized aggressive and chronic periodontal diseases

Débora Heller [a,b], *Carina Maciel Silva-Boghossian* [a,b], *Renata Martins do Souto* [b], *Ana Paula Vieira Colombo* [b,*]

[a] *Department of Dental Clinic, Periodontics, Dental School, Federal University of Rio de Janeiro, Brazil*
[b] *Department of Medical Microbiology, Institute of Microbiology, Federal University of Rio de Janeiro, RJ, Brazil*

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ABSTRACT

Objective: The aim of this study was to distinguish between generalized aggressive (GAgP) and chronic periodontitis (CP) based on the subgingival microbial profiles predominant in these diseases.

Methods: Two-hundred and sixty subjects, 75 with GAgP and 185 with CP were recruited. Full-mouth clinical measurements were recorded. Individual subgingival plaque samples were taken from 7 sites per subject and analyzed for the prevalence and levels of 51 species by chequerboard. Differences between groups were examined by the Mann–Whitney test. Associations between bacterial species and GAgP were examined by logistic regression analysis.

Results: *Actinomyces gerensceriae*, *Actinomyces israelli*, *Eubacterium nodatum* and *Propionibacterium acnes* were detected in significantly greater counts in GAgP, whereas *Capnocytophaga ochracea*, *Fusobacterium periodonticum*, *Staphylococcus aureus* and *Veillonella parvula* were more predominant in CP patients (adjusted $p < 0.001$). *E. nodatum* (at mean levels $\geq 4 \times 10^5$) increased significantly the probability of a subject being diagnosed with GAgP than CP (OR 2.44 [0.96–6.20]), whereas *P. gingivalis* (OR 0.34 [0.11–0.93]) and *T. denticola* (OR 0.35 [0.11–0.94]) were associated with CP.

Conclusions: Very few subgingival species differed in prevalence and/or levels between GAgP and CP in this sample population. In particular, *E. nodatum* was strongly related to GAgP, whereas *P. gingivalis* and *T. denticola* were associated with CP.

1. Introduction

Over the last decades, there has been an enthusiastic discussion on the diagnosis and classification of periodontal diseases.[1–3] During this time, many classification systems based on clinical symptoms have been proposed. However, practitioners find that the clinical diagnosis is not always clear cut, and that clinical signs alone may not distinguish different types of disease. Although the last classification system was based on the Infection/Host Response paradigm,[1] knowledge to diagnose periodontal diseases on an etiologic basis is still limited.[3] Consequently, laboratorial methods with diagnostic potential including microbiological tests,[4] analysis of host-related factors[5] and genetic parameters[6] have been developed.

Although the microbiota associated with periodontal health and disease has been extensively described,[7–9] identification of microbial profiles that could distinguish different

* *Corresponding author at*: R. Gal. Dionísio, 60/604, Humaitá, Rio de Janeiro, RJ, CEP: 22271050, Brazil. Tel.: +55 21 2537 6815; fax: +55 21 2537 6815.
E-mail address: apcolombo@micro.ufrj.br (A.P.V. Colombo).
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forms of disease is inconclusive.[10] The high complexity of the periodontal microbiota, as well as its great inter- and intra-individual variability may account for these difficulties.[11,12] Moreover, there is evidence that differences in the distribution of periodontal pathogens do exist amongst distinct populations.[13–15] Another complication is the fact that most of these pathogenic species are members of the normal periodontal microbiota.[16] Therefore, the presence of periodontal pathogens at low levels in periodontally healthy individuals is a common finding.[17–20] These particular characteristics of periodontal infections emphasize the importance of the development of a more definitive microbial diagnosis which can assist the clinical diagnosis and, consequently, provide a better treatment plan.[4,21] The current study aimed to determine the microbial profiles of subjects clinically diagnosed as having chronic or generalized aggressive periodontitis in an attempt to distinguish between these two forms of disease.

2. Materials and methods

2.1. Subject population

During 2005–2010, a total of 260 patients diagnosed as having generalized aggressive (GAgP) or chronic periodontitis (CP) were recruited from a pool of patients referred to the Division of Graduate Periodontics of the School of Dentistry at the Federal University of Rio de Janeiro (UFRJ), Brazil. All subjects were ≥18 years of age, had ≥14 teeth, and were diagnosed according to criteria described by the American Academy of Periodontology (AAP).[1] Seventy-five subjects were diagnosed as having GAgP based on the following criteria: ≤35 years of age; systemically healthy; history of familial aggregation (if available); presence of at least 3 sites on 3 different teeth (other than central incisors or first molars) with a probing depth (PD) ≥6 mm and clinical attachment level (CAL) ≥5 mm, and bleeding on probing (BOP). One hundred and eighty-five subjects were diagnosed with generalized CP when they presented >30% of sites with PD and/or CAL ≥4 mm, and BOP. Exclusion criteria included systemic conditions that could affect the progression or treatment of periodontal diseases, long-term administration of anti-inflammatory medication, periodontal treatment and/or use of antibiotics in the last 6 months; pregnancy, and nursing. All subjects were informed about the nature of the study and a signed consent form was obtained from each individual. The study protocol was approved by the Review Committee for Human Subjects of the Clementino Fraga Filho University Hospital of the UFRJ (# 1361/2003).

2.2. Clinical measurements

Clinical measurements were performed at 6 sites per tooth (mesiobuccal, buccal, distobuccal, distolingual, lingual and mesiolingual) at all teeth excluding third molars, and included: dichotomous measures of visible plaque, suppuration and BOP, as well as PD (mm) and CAL (mm) recorded to the nearest millimetre using a North Carolina periodontal probe (Hu-Friedy, Chicago, IL). The clinical examinations were performed by two trained and calibrated examiners. The intra-class correlation coefficient for CAL at the site ranged between 0.90 and 0.97, and for PD ranged between 0.90 and 0.94. Data about age, gender, and smoking habit were also obtained. Smoking status was recorded as never-smoker and smoker (current or former smokers).

2.3. Microbiological assessment

Microbial analyses were performed by the chequerboard DNA–DNA hybridization technique,[22] with modifications. In brief, individual subgingival plaque samples were taken from 3 sites with PD ≤3 mm and 4 sites with PD ≥4 mm and BOP per subject. The supragingival plaque was removed and subgingival samples were taken with individual sterile Gracey curettes (Hu-Friedy). The samples were placed in individual eppendorf tubes containing 150 μl of TE buffer (10 mM Tris–HCl, 0.1 mM EDTA, pH 7.6). Samples were lysed by adding 150 μl of 0.5 M NaOH, boiled for 10 min, neutralized with 800 μl of 5 M $C_2H_3O_2NH_4$ and fixed in individual lanes on a nylon membrane (GE Healthcare Life Sciences, Piscataway, NJ) using the Minislot 30 (Immunetics, Cambridge, MA). The Miniblotter 45 apparatus (Immunetics) was used to hybridize 42 whole genomic DNA probes (Table 1). The probes were labelled with digoxigenin using the ''Random Primer Digoxigenin Labelling Kit'' [f]. DNA from serotypes a, b and c of *Aggregatibacter actinomycetemcomitans* was pooled in one probe, as well as *Propionibacterium acnes* serotypes I and II. Bound probes were detected using anti-digoxigenin phosphatase-conjugated antibody (Roche Molecular Systems, Alameda, CA) and fluorescence (GE Healthcare Life Sciences) by an imaging capture system (GE Healthcare Life Sciences). Signals were evaluated visually by comparison with the standards at 10^5 and 10^6 cells for the test species on the same membrane. They were recorded as: 0 = not detected; 1 = $< 10^5$ cells; 2 = $\sim 10^5$; 3 = 10^5–10^6 cells; 4 = $\sim 10^6$; 5 = $> 10^6$ cells. The sensitivity of this assay was adjusted to permit detection of 10^4 cells of a given species by adjusting the concentration of each DNA probe. This procedure was carried out in order to provide the same sensitivity of detection for each species. Failure to detect a signal was recorded as zero, although conceivably, counts in the 1–1000 range could have been present.[23]

2.4. Statistical analysis

A statistical programme (except 'program' in computers) (SPSS, Statistical Package for the Social Sciences, version 17.0, Chicago, IL) was used for all the analyses. Differences in mean clinical parameters between GAgP and CP subjects were determined by the Mann–Whitney and χ^2 tests. Microbiological data were expressed as median levels of each species. The levels (scores 0–5) of each species in a sample were converted to absolute numbers, computed for each subject, log transformed and then averaged across subjects in the groups. Significance of differences in levels of each subgingival species between groups was determined by the Mann–Whitney test. Adjustment for multiple comparisons was made as described by Socransky et al.[24] In brief, an overall p of $0.05 = 1 - (1 - k)$[42] was computed where k was the desired individual p value. Thus, from this computation, a p value ≤0.0011 was considered to be statistically significant at $p < 0.05$. Demographic,