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Presence of *Actinobacillus actinomycetemcomitans* on the community Periodontal Index (CPI) Teeth in Periodontally Healthy Individuals

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Abstract

The aim of this study was to compare the prevalence of *Actinobacillus actinomycetemcomitans (A. actinomycetemcomitans)* located at Community Periodontal Index (CPI) teeth versus randomly selected teeth to determine if the CPI teeth are representative of this bacteria subgingival colonization in periodontally healthy young individuals. Forty-four individuals between 18 and 27 years of age were included in the study (mean age 23.11 ± 2.91 years). Pooled subgingival plaque samples were collected with paper points from the mesio-buccal aspect of the CPI teeth (10 teeth for adults and 6 teeth for persons under 20 years of age) and transported in reduced Ringer's solution to the culture medium. Sixty days following the first microbial analysis, new pooled microbial samples were obtained from the mesio-buccal aspect of 10 or 6 randomly selected teeth. The presence of *A. actinomycetemcomitans* was determined using a selective culture medium. Microbiological data were assessed by the Wilcoxon test ($p < 0.05$). A border line significance ($p=0.51$) was observed between CPI teeth and randomly selected teeth in terms of detecting the subgingival occurrence of *A. actinomycetemcomitans*. CPI teeth showed to be representative of *A. actinomycetemcomitans* subgingival colonization. Therefore, these results suggest that in periodontally healthy young individuals, CPI teeth could be an appropriate source of samples for the subginginval detection of this pathogen.

Keywords: Community periodontal index, CPI, periodontal diseases, prevalence, culture media, *Actinobacillus actinomycetemcomitans*

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Introduction

The Community Periodontal Index (CPI)[1] has been widely used in an attempt of standardizing the diagnosis in terms of periodontal epidemiology. This index takes into consideration ten teeth in the oral cavity [2 (17), 3 (16), 8 (11), 14 (26), 15 (27), 18 (37), 19 (36), 24 (31), 30 (46), and 31 (47)] and evaluates the occurrence of gingival bleeding, presence of supra- and subgingival calculus, periodontal pockets with probing depths between 3.5 and 6.0 mm, as well as clinical attachment loss. For individuals under the age of 20 years, only six teeth were examined; third molars were excluded. This modification is made in order to avoid scoring the deepened sulci associated with eruption as periodontal pockets. For the same reason, when examining teenagers or children under the age of 15 years, pockets should not be recorded, i.e., only bleeding and calculus should be considered. Clinical and epidemiological trials have used the CPI to determine periodontal status as well as the need for treatment of target populations.[2-7] Although this system analyzes a limited number of teeth, it has shown to be representative of full mouth records. For this reason the CPI is useful in periodontal research, especially to reduce the time needed for examinations when the study population comprises a large number of individuals.[8] In addition this method allows the elaboration of preventive and therapeutic programs as well as the quantification of biological and environmental risk factors related to the disease onset and progression.[3]

The concept that specific bacteria play an etiologic role in periodontal diseases resulting in clinical attachment loss and alveolar bone loss is well established and widely accepted.[9, 10] Several microbiological studies have associated periodontal diseases with different periodontal pathogens or groups of bacteria, such as fusobacteria, spirochetes, *Actinobacillus actinomycetemcomitans (A. actinomycetemcomitans), Tannerela forsythensis, Porphyromonas gingivalis, Prevotella intermedia, Campylobacter rectus*, and others.[10-14] The substantial occurrence of *A. actinomycetemcomitans* in individuals with aggressive periodontal disease, and the increase of bacterial levels according to disease severity, can be partially explained by the wide range of virulence factors featured by this microorganism, especially the leukotoxin production.[15-17]

There is no agreement in the literature about number or group of teeth that should be sampled to establish specific periodontal microbiota.[6, 10, 16, 18-21] Muller et al.[22] suggested the analysis of the deepest pockets of every quadrant of the dentition. In a longitudinal study Tanner et al.[7] showed that samples taken from one to five gingivitis and one to five healthy sites could be satisfactory to compare the subgingival microbiota of different periodontal status using culture and DNA probe assays. Nevertheless, most of the studies include the assessment of incisors and molar teeth in young individuals, due to their higher exposure to the aggressive periodontitis (formerly Juvenile Periodontitis), primarily in their local form.[13, 19, 23, 24, 25, 26] Savitt et al.[27] reported clinical criteria reduced the minimum number of sites required to detect a pathogen. In the presence of 4 mm probing depth, in addition to bleeding on probing, two sites were required for a good confidence level of finding infected sites. Müller et al.[28] examined the presence of *A. actinomycetemcomitans* in a total of 1005 pooled subgingival samples obtained from the first molars of 201 individuals between 18 to 25 years old. Yuan et al.[29] analyzed subgingival samples taken from the mesial aspect of two randomly selected permanent first molars from 328 primary school children.

Therefore, the aim of this study was to compare the prevalence of *A. actinomycetemcomitans* located at CPI teeth versus randomly selected teeth to determine if the CPI teeth are representative of the bacteria subgingival colonization in periodontally healthy young individuals.

Materials and Methods

Subject and Site Selection

Forty-four periodontally healthy young adults, between 18 and 27 years of age (mean age 23.11 ± 2.91 years), presenting all permanent teeth (excluding third molars) were included in this study. The periodontal status was established according to clinical parameters proposed by the World Health Organization (WHO)[1] and assessed with a 0.5 mm ball tip periodontal probe with black band markers at 3.5 mm, 5.5 mm, 8.5 mm, and 11.5 mm (PCP 11.5BBR, Hu-friedy). Exclusion criteria included patients with periodontal disease, diabetes, immunocompromised individuals, pregnant and breast-feeding women, individuals with orthodontic devices, or who had taken antibiotics within 6 months prior to the clinical examination.

All subjects signed an informed consent which was previously approved by the Institutional Committee on Research Involving Human Subjects.

Subgingival Plaque Sample Collection and Culture

Pooled microbial samples were first obtained from the mesio-buccal aspect of the CPI teeth (Figure 1).

For persons to 20 years of age, 10 teeth were sampled; while 6 teeth were sampled for individuals under the age of 20 years. According to age, sixty days following the first collection, new pooled subgingival plaque samples were taken from the mesio-buccal aspect of 10 or 6 randomly selected teeth. Excluding third molars, a table of random numbers was used to select 10 or 6 teeth out of the 30 possible teeth which represented the entire dentition. The periodontal sites were isolated with cotton rolls and the supragingival plaque was removed with sterile cotton pellets. The microbiological samples were taken using two sterile paper points. The paper points were inserted to the depth of the mesio-buccal aspect and kept in position for 60 s. The paper points were placed into the same micro-tube containing 1.5 ml reduced Ringer's solution and kept at 4°C until the processing procedure was carried out. The processing procedure was performed within the next 2 hours. The samples were mixed well by vortex for sixty seconds; 0.1 ml of each of the samples was plated, in duplicate, onto trypticase soy bacitracin vancomycin (TSBV) agar plates, containing 1g/l yeast extract, 100ml/l horse serum, 75mg/l bacitracin, and 5mg/l vancomycin.[30] The plates were incubated at 37°C for 5 days in an environment containing 5% CO^2. Colonies of *A. actinomycetemcomitans* were identified by morphology in stereoscopic microscopy. Small, rough, round, convex, translucent, and adherent colonies with stellate central structure were considered positive. In order to confirm the results Gram staining, biochemical tests of glucose, fructose, and manose fermentation, as well as catalase reaction were performed for the positive colonies.[31]

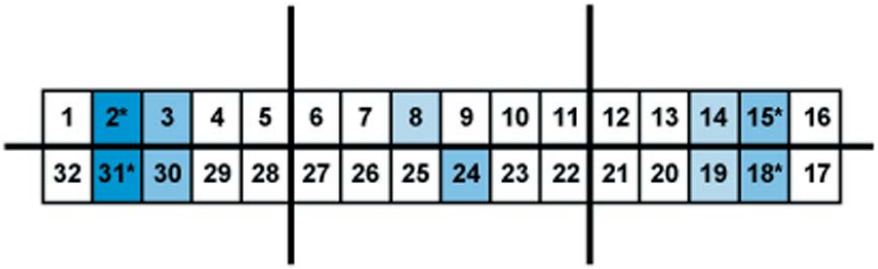

Figure 1. CPI teeth.
(*Teeth not considered to clinical evaluation under 20 years of age.)

Statistical Analysis

The difference between pathogen detection using CPI teeth and randomly selected teeth as well as the influence of age in the presence of *A. actinomycetemcomitans* was assessed using the Wilcoxon test, with the significance level being established at 5% ($p < 0.05$). The association between age and the presence of bacteria was analyzed considering there were two different age categories. These categories were previously determined according to the median value of age (23 years of age) as the dividing factor.

Results

Forty-four young adults were included in this present study, 12 (27%) males and 32 (73%) females. These periodontally healthy individuals presented with all permanent teeth (excluding third molars) and showed probing depth < 3.0 mm, clinical attachment level < 2.0 mm, and absence of either gingival bleeding or supra- and subgingival calculus.

Six of the individuals sampled, who account for 14% of study population, showed positive detection for *A. actinomycetemcomitans*. Four of the 6 positive samples (67%) were obtained from CPI teeth and 2 (33%) from randomly selected teeth (Figure 2).

The presence of *A. actonomycetemcomitans* between CPI teeth and randomly selected teeth showed a border line significance ($p = 0.051$).

Although the random teeth were selected from all permanent teeth using a table of random numbers, in the study population the random teeth were never the same as the CPI teeth. For this reason, the first and second pooled microbial samples comprised different sites. (Figure 3)

There was no association between age and occurrence of *A. actinomycetemcomitans*. Among the positive individuals, one was 19 years old and 3 were 22 years old; the other two positive individuals were 26 and 27 years old, respectively. Distribution of positive individuals regarding their age is expressed in Figure 4.

Discussion

There is a wide range of criteria to establish which index or indexes are capable of determining the real features of periodontal

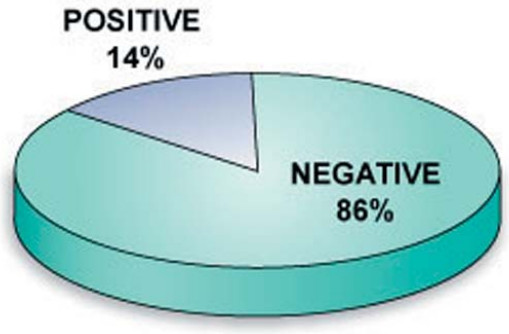

Figure 2. Distribution of the study population (n=44) with the subgingival presence (positive) of *A. actinomycetemcomitans*.

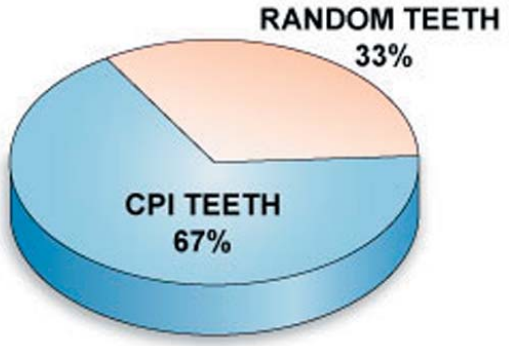

Figure 3. Percentage of individuals with positive samples for *A. actinomycetemcomitans* in 10 randomly selected teeth or CPI teeth.

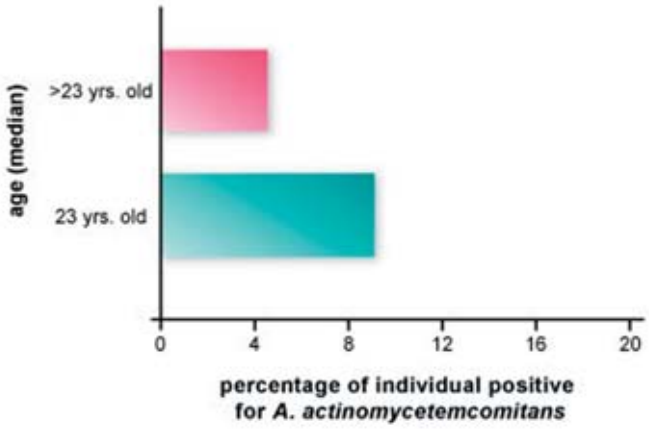

Figure 4. Distribution of positive individuals (%) for *A. actinomycetemcomitans* according to age groups established by median of age.

disease. These criteria include different standards in measuring probing depth, clinical attachment loss, presence of plaque and calculus, gingival bleeding, and microbiological evaluation. Partial indexes usually take into consideration a certain number of teeth, which present features that are common to all teeth in the oral cavity as well as the susceptibility of selected teeth to referred pathologies.[8] Although the CPI was not developed for microbiological studies, the teeth selected by this method appear to be appropriate for *A. actinomycetemcomitans* detection. This is especially true for young people, since it includes the permanent incisors and molar teeth (Figure 1).

Suda et al.[32] chose the mesio-buccal aspect of the maxillary right first molar as a sampling site. Furthermore, Kamma et al.[33] just isolated *A. actinomycetemcomitans* in permanent and decidious molars. The inclusion of incisors and molars within research protocols[34], which evaluate young individuals, is justified by the clinical characteristics of aggressive periodontitis.[23-25] It must be said studies which isolated *A. actinomycetemcomitans* from incisors and molar teeth were generally performed in subjects with aggressive periodontitis. The purpose of this study was to evaluate the efficacy of CPI teeth, which is already widely used for clinical studies, in the search of a periodontal pathogen, *A. actinomycetemcomitans*. This was done to minimize possible sampling errors and to provide additional information on an increasing risk for periodontal disease from certain bacterial exposure.

In this study the prevalence of *A. actinomycetemcomitans* in periodontally healthy individuals was 14%, which is in agreement with previous studies conducted in different populations.[27, 32, 35-38] Asikainen et al.[35] and Clerehugh et al.[36] showed a prevalence of 18% of this pathogen in teenagers either with a healthy periodontium or minimum presence of periodontal disease. In an earlier study performed by our group Cortelli et al.[39] detected 19.3% of *A. actinomycetemcomitans* in Brazilian individuals diagnosed with either moderate or severe periodontitis. Takeuchi et al.[40] observed a relatively low prevalence of *A. actinomycetemcomitans* (17.5%) in generalized aggressive periodontitis. Futhermore in saliva samples of Sudanese adults with ages ranging between 19 to 53 years, Darout et al.[37] found 16.1% of the individuals with high levels of this pathogen.

Pooled subgingival plaque samples were cultured for the detection of *A. actinomycetemcomitans*. According to Boyer et al.[41] paper point samples may be collected from multiple sites and pooled as a single test to yield a patient assessment of bacterial colonization. Furthermore, in the present study, a control of randomly selected teeth was utilized that incorporated periodontal sampling schemes proposed by different authors.[21-26, 32, 40]

When the presence of *A. actinomycetemcomitans* was comparatively evaluated between CPI (67%) and randomly selected (33%) teeth, a border line significance (p= 0.51) was observed among individuals. Due to the similar results observed between CPI teeth and randomly selected teeth, pooled subgingival samples obtained from CPI teeth could yield the *A. actinomycetemcomitans* infection in a patient level.

Of interest was the fact no individual with *A. actinomycetemcomitans* positive sample in the first collection (CPI teeth) showed a positive result in the second collection (randomly selected teeth). Although a table of random numbers was used, the first and second pooled microbial samples evaluated different sites in the study population. Considering the teeth sampled were different, these results support the concept of site-specificity and show the difficulty of a new microbial species colonizing in sites that already present an established microbiota.[10, 42] Excluding third molars, the randomly selected teeth were chosen from the entire dentition. According to this sampling method each CPI tooth could be selected as a random tooth for a given subject. For this reason, a sixty-day period between each microbial collection was established. Moreover, this time period took in consideration the low levels of bacteria usually observed in periodontally healthy individuals[7, 15, 16, 17, 24]

In the present study an association between age and pathogen occurrence was not observed. The inclusion of teenagers and young adults, up to 27 years of age, may have shown a higher frequency of A. actinomycetemcomitanes in younger individuals. Cortelli et al.[16] studied the occurrence of *A. actinomycetemcomitans* in individuals between 14 and 76 years of age and found a positive correlation between bacterial strains and subjects under 28 years of age.

Few bacterial species have been suggested as risk factors for periodontal disease. Since *A. actinomycetemcomitans* is considered one of these species[10, 14, 15], it is relevant to remember its pathogenicity potential, which is associated with its virulence factors, such as production of lipopolyssaccharides, bacteriocines, cytosines, collagenase, leukotoxin, and tissue invasion.[9, 15, 16, 43] According to these concepts, periodontally healthy individuals may feature a higher risk of developing periodontal disease when exposed to this pathogen. Therefore, the individuals who hosted *A. actinomycetemcomitans* in the present study might have an increased risk to develop periodontitis, considering both the presence of the microorganism at CPI teeth or at randomly selected teeth.

Conclusions

In periodontally healthy young individuals, CPI teeth showed to be representative of *A. actinomycetemcomitans* subgingival colonization. For this reason, CPI teeth could be an appropriate source of samples to detect the subginginval presence of this bacteria.

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