Community Dental Health (2011) **28,** 301–304
Received 18 August 2009; Accepted 19 April 2010

doi:10.1922/CDH_2592Cogo04

Prevalence of periodontopathogens in a black Brazilian secluded community matched with a black urban population

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Objective: To evaluate the prevalence of periodontopathogens according to periodontal profile in a black Brazilian secluded community matched with an urban black population. ***Participants:*** A total of 84 subjects were selected, 42 (mean age 25.7 sd 18.0 years) from a secluded community called Santo Antonio do Guapore (SAG) and 42 (mean age 25.4 sd 18.1 years) from an urban area of Sao Paulo State (SPT). ***Methods****:* Participants received clinical examinations as follows: periodontal pocket depth; clinical attachment loss; plaque and gingival indexes. After examination, the secluded population was classified as periodontal health (13), gingivitis (15) or periodontitis (14). Then, 182 urban volunteers were screened and 42 subjects were selected matched for the variables: periodontal diagnosis, age (±2 years) and gender. Samples were taken for microbial analysis. Genomic DNA for *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Campylobacter rectus, Tannerella forsythia* and *Prevotella intermedia* was provided by polymerase chain reaction. ***Results:*** Except for *C. rectus*, all pathogens were present in both groups with no statistically significant difference. In particular, *C. rectus* was more prevalent only in gingivitis subjects from the SPT group ($p<0.05$). A high frequency of periodontopathogens was related to the severity of periodontal disease. ***Conclusion:*** In general, the prevalence of the examined periodontopathogens in this study did not differ between a secluded black Brazilian population and an urban black population.

Key Words: *periodontitis, ethnic groups, polymerase chain reaction, bacteria.*

Introduction

In general, systemic and oral health are poorer among racial and ethnic minorities (Byrd and Clayton, 1992). Typically, epidemiological data regarding levels of oral diseases are based on national samples. Due to the limited number of blacks in most of these studies, analysis regarding blacks is often restricted to overall descriptions of major oral diseases and conditions and oral hygiene practices, rather than descriptions of the diversity within the black populations (Cherry-Peppers *et al.* 1995).

The black community of Santo Antonio do Guapore (SAG) was founded in the 18th century by escapees from the the slave work imposed to them by the authorities. It is located southeast of Rondônia, Brazil, bordering Bolívia (Teixeira and Fonseca, 2010). The community has survived in isolation and is only accessible through lengthy travel by boat. According to reports from elders, as well as from notes taken from chronicles and travelers, it is estimated that today's local population is about 70; it has never exceeded 400 (Teixeira and Fonseca, 2010).

We hypothesized that the isolation from the modern world would be accompanied by a higher prevalence of the target periodontal bacterial species. Then, a study of bacterial prevalence could evaluate whether the microbial profile, according to periodontal conditions, of a secluded community would differ from that of an urban black community living in Sao Paulo State (SPT), when these groups' subjects were individually matched by periodontal profile, age and gender.

Methods

Data and personal information regarding medical and dental histories of the subjects were obtained from their responses to a questionnaire. All subjects signed an informed consent form previously approved by the Institutional Committee on Research Involving Human Subjects of the Faculdade São Lucas (protocol AP/CEP/138/07). Participants were recruited in SAG and SPT between June 2007 and December 2008. Clinical measurements and microbial samples were collected at SAG by a trained and calibrated examiner (JD) in a rudimentary chair under natural light. Given the remote location, the clinical measurements and microbial sample collection at SPT were performed by another examiner (JRC) trained at the Department of Dentistry at the University of Taubate. To determine the periodontal status of the subjects, the clinical examination comprised visible plaque index and gingival bleeding index, periodontal probing depth and clinical attachment level. Their periodontal status was determined following the criteria defined by the American Academy of Periodontology (Armitage, 1999). After examination, the SAG population was classified as

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periodontally healthy, gingivitis or periodontitis. Then, 182 urban volunteers were screened and 42 subjects were selected to match three variables: periodontal diagnosis, age (sd 2 years) and gender.

After supragingival biofilm removal, a pooled subgingival sample was collected (as described by Cortelli *et al.*, 2008) from each subject, from the primary or permanent first molars and central incisors. For subjects missing those teeth, microbial samples were obtained from primary or permanent second molars and/or lateral incisors. The bacterial cells in the microtube were dispersed using a Vortex and centrifuged for 3 minutes at 12,000 rpm. From the cellular bacteria pellet, genomic DNA was extracted using a commercial DNA purification Kit (InstaGene®, Bio-Rad laboratories, Hercules, CA, USA) according to the manufacturer's instructions.

The presence of *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Campylobacter rectus, Tannerella forsythia* and *Prevotella intermedia* were determined by polymerase chain reaction (PCR). The bacterium-specific primer (5'-3') sequences used in this study were: *A. actinomycetemcomitans* – forward primer - 5'AAACCCATCTCTGAGTTCTTCTTC-3', reverse primer - 5'-ATGCCAACTTGACGTTAAAT-3', (Amplicon length - 557 bp); *P. intermedia* – 5'-TTTGTTGGGGAGTAAAGCGGG-3', 5'-TCAACATCTCTGTATCCTGCGT-3', (575 bp); *P. gingivalis* - 5'-AGGCAGCTTGCCATACTGCG-3', 5'-ACTGTTAGCAACTACCGATGT-3', (404 bp); *T. forshytia* - 5'-GCGTATGTAACCTGCCCGCA-3', 5'-TGCTTCAGTGTCAGTTATACCT-3' (641 bp); *C. rectus* - 5'- TTTCGGAGCGTAAACTCCTTTTC-3', 5'-TTTCTGCAAGCAGACACTCTT-3', (598 bp); Universal- 5'GATTAGATACCCTGGTAGTCCAC3', 5'CCCGGGAACGTATTCACCG3', (602 bp). To confirm the presence of bacterial DNA in clinical samples, a ubiquitous primer was used as described by Ashimoto colleagues (1996).

A total volume of 25 µL of the PCR mixture contained 10 µL of the DNA sample, 2.5 µL of a 10x PCR buffer (Invitrogen, Carlsbad, CA, USA), 1.25 units of Taq DNA polymerase (Invitrogen), 0.2 mM of each deoxyribonucleotide (Invitrogen), 1.5 mM of $MgCl_2$ and 1.0 µM of each primer.

The PCR amplification was performed in a Mastercycler Gradient thermal cycler (Eppendorf, Westbury, NY, USA) using a standard protocol that includes an initial denaturation step at 95ºC for 5 minutes, followed by 35 cycles of denaturation at 95ºC for 30 seconds, an annealing step at 55ºC for 30 seconds, and an extension step at 72ºC for 1 minute, with a final extension period of 72ºC for 5 minutes.

The PCR products were analyzed by electrophoresis (5V/cm^2) in 1.5% agarose gel, stained with ethidium bromide (0.5 µg/ml), and photographed under ultraviolet light. PCR fragments were compared with both negative and positive control DNA isolated from standard bacterial strains in order to verify the primer specificity and identify DNA contamination.

The frequencies of bacterial species in SAG and SPT individuals were analyzed using the Chi-Square test. All tests were performed using the statistical software SPSS 13.0. Results were considered statistically significant at p-values <0.05.

Results

Included in the study were 84 individuals with mean age 25.5 sd 18.0 years, range 7-73, 42 (mean age 25.7 sd 18.0 years) in the SAG and 42 (mean age 25.4 sd 18.1 years) in the SPT group. The population consisted of 24 males, 18 females with the same age range, 7-73. Table 1 shows the distribution of the study population according to periodontal diagnosis.

Inter-examiner reliability was tested by means of the weighted Kappa statistics (Cohen, 1968). The weighted Kappa statistics for the periodontal probing depth scores ranged from 0.78 to 0.86.

Both groups showed evidence of colonization by all of the studied bacteria. Intra-group analysis of pathogen prevalence is displayed in Figure 1. *C. rectus* was more prevalent in the SPT group than the SAG group ($p < 0.05$) and no difference between groups was observed in the prevalence of *P. gingivalis*, *P. intermedia,* and *T. forsythia. A. actinomycetemcomitans* was found at a prevalence of less than 30% in both groups.

Figure 2 shows the frequency of all pathogens according to periodontal diagnosis. *C. rectus* was also more prevalent in gingivitis subjects from the SPT group ($p < 0.05$) as compared to the SAG group. Also, in general, the frequency of all bacteria increased according to severity of disease, from periodontal health to periodontitis.

Table 1. Distribution of SAG and SPT populations according to periodontal diagnosis

		SAG	*SPT*
	Males, Females	*Number (mean age, sd) age range*	*Number (mean age, sd) age range*
Periodontal Health	8, 5	13 (8.30, sd 1.97) 7 – 12 years	13 (7.92, sd 2.01) 7 - 11 years
Gingivitis	9, 6	15 (25.87, sd 11.37) 15 - 50 years	15 (25.47, sd 12.58) 15 - 50 years
Periodontitis	7, 7	14 (41.64, sd 17.23) 25 - 73 years	14 (41.43, sd 17.24) 25 - 73 years

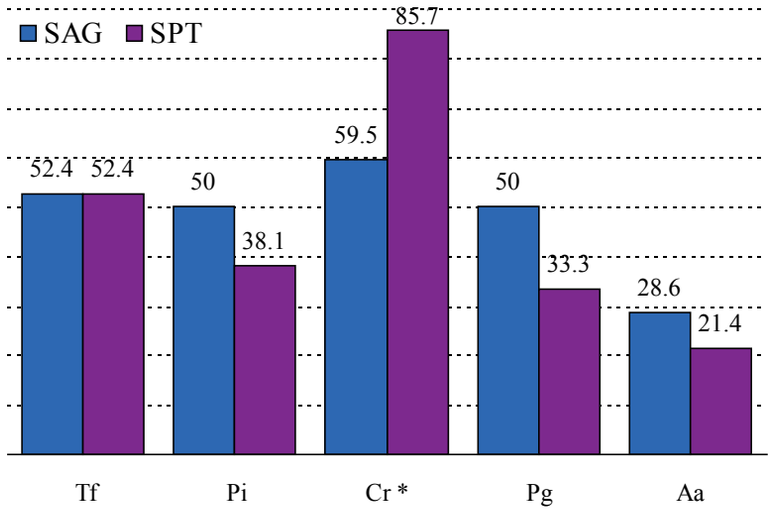

Figure 1. *Frequency of T. forsythia, P. intermedia, C. rectus, P. gingivalis and A. actinomycetemcomitans in SAG and SPT groups*

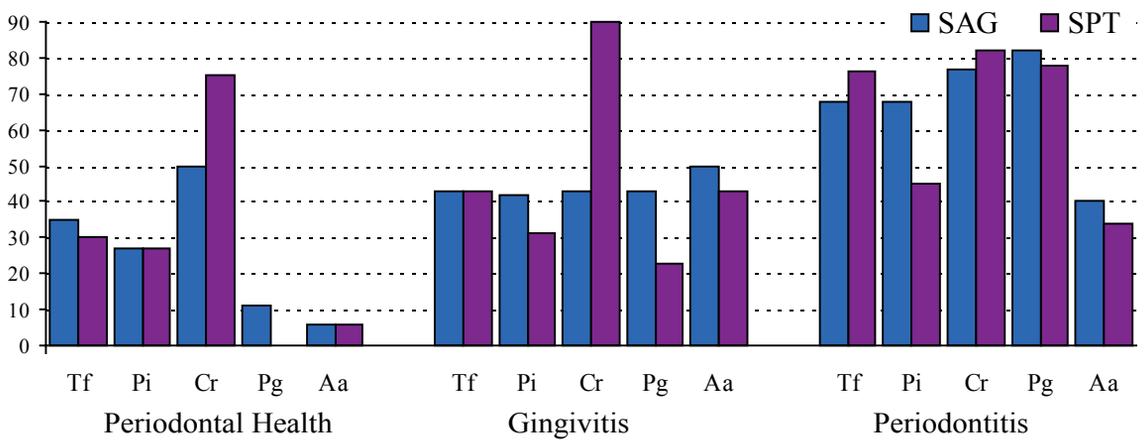

Figure 2. *Frequency of T. forsythia, P. intermedia, C. rectus, P. gingivalis and A. actinomycetemcomitans in SAG and SPT groups according to periodontal status*

Discussion

This is a pioneer study of the SAG community and which searched for any differences between contrasting communities, a secluded one and an urban one. In a review paper Gjermo *et al.* (2002) stated that only a few representative epidemiological studies related to periodontal conditions are available in Latin America. There were no previous dental studies published for any any community in the State of Rondônia.

Today, the SAG community includes 67 inhabitants; 72% of whom were initially screened. However, 6 were excluded being unable to leave work to attend data collection and a further two did not consent to participate; therefore, 42 were examined. The intention was to examine every person available in the community because besides the evaluation of clinical and microbial parameters, general dental clinical maintenance care as instructions about oral hygiene habits, atraumatic restorative treatment, exodontias, supra- and subgingival scaling root planing were performed.

This study hypothesized that the isolation from the modern world would be accompanied by a higher prevalence of the target periodontal bacterial species. However, the hypothesis was not supported because only *C. rectus* was significantly more prevalent when SAG community was compared to the SPT population.

When comparing the frequency of each bacterial species we observed only a tendency for the SAG community to show a higher number of subjects harboring the target bacteria than the SPT population. However, an inference based on statistical evidence was not possible since the sample size *n* was insufficient for effective statistical treatment of the data, even with the authors having examined almost all the people. There are no similar studies with which to compare these findings. Two studies analyzed the prevalence of periodontal bacteria in isolated populations but they did not make comparisons with the similar ethnicity urban groups. Vieira *et al.* (2009) studied the prevalence of *A. actinomycetemcomitans* in native Brazilians from Umutina Reservation, and showed that this bacterial species was more prevalent in chronic periodontitis subjects than in gingivitis subjects. This result correlates with the present study, since both populations showed periodontally healthy subjects presenting a lower bacterial prevalence than subjects with gingivitis.

In addition, subjects with gingivitis presented lower frequencies than subjects with a diagnosis of periodontitis. However, contrary to our findings, no association between periodontal status and presence of periodontal pathogens was found in a rural community of natives in Guatemala, Central America (Dowsett *et al.*, 2002).

When we analyzed the data from the present study, we performed a deeper analysis of a previous study on black people conducted by our group in the city of Salvador, Bahia (Victor *et al.*, 2008). Overall, prevalence of *A. actinomycetemcomitans*, *P. gingivalis, E. corrodens* and *F. nucleatum* was high in the studied population. Nevertheless, this high prevalence was not related to the periodontal status. Most subjects presented healthy periodontal conditions and the most severe periodontal disease was gingivitis. A possible explanation for the fact that high bacterial prevalence does not necessarily lead to "severe disease" is based on the quality of the response of the host to the biofilm. Furthermore, as reported by Haffajee *et al.* (2006), the difference between a healthy and a disease condition can be related to the proportion of species present, such as *T. forsythia*, *P. gingivalis* and *T. denticola*. Therefore, our data supports few but interesting findings: the carriage of the putative pathogens in an isolated community was similar to an urban matched population; and the severity of periodontal disease did not direct correlate with the mere presence of these pathogens. Then, disease might be related more to the numbers and activity of the pathogens, or to the immune status of the host. *A. actinomycetemcomitans*, *P. gingivalis* and *T. forsythia* were searched because they are considered true periodontal pathogens due to evidences from longitudinal studies (Ezzo and Cutler, 2003 for details). In addition, *C. rectus* and *P. intermedia* were selected because they are members of the orange complex that is related to moderate levels of disease.

We concluded that although the population of SAG lives under worse conditions including sanitary conditions and lacks access to medical and dental treatment these do not increase the presence of the periodontopathogens when compared with a matched urban black population.

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