Periodontal Pathogens and Clinical Periodontal Status of School Children: A Cross-Sectional Study

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Introduction

Periodontal disease (PD) has a multifactorial etiology and initiates by the accumulation of dental biofilm that affects tissues surrounding. Its pattern, severity and progression are determined by social, systemic, genetic and microbial composition among other risk factors [1]. The most prevalent type of periodontal disease worldwide is plaque-induced gingivitis which shows reversibility as a key characteristic [2].

Epidemiological data have shown that plaque-induced gingivitis affects, in different rates, dentate populations in all ages [3], but mainly children and adolescents [4,5]. Jaleeddin and Ramezani [6] found a prevalence of 97-98% of gingivitis in children aged from 6 to 9. According to the United Nations Organization in 2010 more than 1.8 billion people were aged between 10 and 25. In Brazil it is estimated that in 2030 19.6% of population will be up to 14 years old, this makes the country vulnerable to gingivitis occurrence [7].

Bacterial species that can cause gingivitis colonize oral cavity since early in life [8] reaching high numbers at the age of 2 [9]. Independent of age, the complexity of the microbiota is often related to the healthy or diseased status of periodontium [10]. Also, presence of some bacterial species may increase the risk for periodontal diseases [11].

Microbial data is clearer in adults than in children, Sakai et al. [12], for example, observed a high percentage of children harboring at least one periodontal pathogenic species (A. actinomycetemcomitans, P. gingivalis, T. denticola), however, Gafan et al. [13] found a higher frequency of T. forsythia in children without disease. Interestingly, it was suggested that children are more resistant to gingivitis than adults in spite of the fact that they show increased subgingival levels of Leptotrichia sp., Capnocytophaga sp., Selenomonas sp. and Bacteroides sp [14]. Cortelli et al. [15] in a sample population of high plaque index reported and association between P. intermedia and gingivitis and between C. rectus and periodontal health.

Therefore, it has been suggested that colonization by pathogens associated with gingivitis happens earlier than previously believed. According to Tanner et al. [16] this knowledge would be helpful for the understanding of disease development and determination of interceptive measures. Although not related to tooth loss, per se, gingivitis is a chronic inflammatory disease that deserves to be controlled aiming at a healthier status for the entire individual. This cross-sectional study compared the frequency of target bacterial species and its relation to periodontal status in school children.

Keywords: Gingivitis; Child development; Periodontal diseases; Bacteria

Abstract

Although gingivitis affects dentate people in all ages it reaches high prevalence levels in children and adolescents.

Purpose: This cross-sectional study compared the frequency of target bacterial species and its relation to periodontal status in children.

Methods: 254 systemically healthy children, between 6 and 12 years of age, with mixed dentition, having a healthy periodontium or gingivitis were selected. Whole-mouth dichotomous plaque and gingival indices were evaluated and microbial samples were collected from tongue dorsum, first molars, right maxillary and left mandibular incisors.

Results: P. gingivalis was the most frequent pathogen in the sulci of periodontally healthy children; T. forsythia and A. actinomycetemcomitans were the less detected species in tongue samples. P. gingivalis was the most frequent pathogen in both teeth and tongue samples among gingivitis children. C. rectus was more frequent in the sulci of healthy children while frequency of P. gingivalis was higher in gingivitis. Conclusions: It can be concluded that P. gingivalis was highly frequent and that C. rectus was more frequent in healthy children. At this range of age clinical status was not always directly related to the presence of the searched pathogens.
Materials and Methods

The present study was reviewed and approved by the Ethics Committee of the University of Taubaté (Protocol #0317/07). Legal guardians or parents signed the informed consent form after verbal and written explanations about study design and procedures.

Inclusion criteria and determination of groups

This convenience sample was composed of 254 systemically healthy children, aged between 6 and 12, with mixed dentition who were divided into two groups according to their periodontal status: periodontally healthy (<30% of periodontal sites showing gingival bleeding); gingivitis (>30% of periodontal sites showing gingival bleeding) [17]. Children who had taken antibiotics in the previous 3 months, had chronic systemic diseases, extensive caries lesions, had no molars and incisors or who wore orthodontic appliances were excluded from the study.

Clinical measurements and diagnosis

Two trained and calibrated examiners measured plaque [18] and gingival [19] indices in a single afternoon visit in the dental unit of a public school. Later, original scores were dichotomized according to absence (0) or presence of any amount of plaque (1) and to absence (0) or presence of gingival bleeding (1). Intra and inter-examiners reproducibility values were tested using Kappa test (K). The examiners were considered calibrated when agreement rates of not less than 90% were reached. Also, a bite-wing X-ray examination was conducted to evaluate the presence of periodontal bone resorption [20].

Microbiological assessment

A single microbial sample was taken from the central 1 cm² area of the dorsum of the tongue of each child, using a swab with reduced Ringer’s solution, rotated six times. Each swab was transferred into a microtube also containing reduced Ringer’s solution (1 ml). After removal of supragingival plaque, a pooled subgingival sample was collected from the mesiobuccal aspect of all first molars and mesial aspect of right maxillary and left mandibular incisors using sterile paper points number 30 (Dentsply, York, PA, United States) inserted into the depth of the gingival sulcus. After being placed in the sulcus for 60 seconds [21], paper points were removed and immediately transported into a microtube containing 1.5 ml of reduced Ringer’s solution (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom) and transported to the laboratory. All samples were kept at -80°C until processing.

The presence of C. rectus, P. gingivalis, A. actinomycetemcomitans, P. intermedia and T. forsythia was determined by polymerase chain reaction (PCR). 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, Carlsbad, CA, USA), 1.5 mM of MgCl₂ and 1.0 μM of each primer.

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

The final products were separated on 1.5% agarose gel, stained with ethidium bromide (0.5 μg/mL) and photographed under ultraviolet light to confirm the existence of the target oral bacterial species. A 100bp DNA ladder (Invitrogen, Carlsbad, CA, USA) was used as the molecular weight marker. Both positive and negative controls were included for the PCR reaction in order to verify the primer specificity and identify and DNA contamination.

Table 1: Bacteria and specific primer sequences.

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Primers</th>
<th>Amplicon</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. rectus</td>
<td>Sense TTTCCGAGCCTAAGCTCTTTTCC-3'</td>
<td>5'- 598 bp</td>
</tr>
<tr>
<td></td>
<td>Antisense TTTCGCAAGCAGACACTTT-3'</td>
<td>5'-</td>
</tr>
<tr>
<td>A. actinomycetemcomitans</td>
<td>Sense AAACCCCATCTGAGTCTTCTC-3'</td>
<td>5'- 550 bp</td>
</tr>
<tr>
<td></td>
<td>Antisense ATGCCAATTGAGCTTAAAT-3'</td>
<td>5'-</td>
</tr>
<tr>
<td>P. intermedia</td>
<td>Sense TTTGTGGGGAGTAAAGCGGGG-3'</td>
<td>5'- 575 bp</td>
</tr>
<tr>
<td></td>
<td>Antisense TCAACATCTCTGTATCCTCGG-3'</td>
<td>5'-</td>
</tr>
<tr>
<td>P. gingivalis</td>
<td>Sense AGGCCAGTTGCCCATACTGCGG-3'</td>
<td>5'- 404 bp</td>
</tr>
<tr>
<td></td>
<td>Antisense ACTGTAGCAACTACCCGATGT-3'</td>
<td>5'-</td>
</tr>
<tr>
<td>T. forsythia</td>
<td>Sense 5'GCGTATGTAACCTGCCGA3'</td>
<td>5'- 641bp</td>
</tr>
<tr>
<td></td>
<td>Antisense TGCTCAGTGTCAGTTTACCT-3'</td>
<td>5'-</td>
</tr>
</tbody>
</table>

Statistical analysis

Clinical data was compared using Mann-Whitney test. The frequencies of the periodontal pathogens were analyzed using the Chi-squared test (χ²). Statistical analyses were performed using statistical software (Bio Estat 5.0 and SPSS11.0) where the statistical significance was established at alpha 5%.

Results

A total of 254 children of both genders were included in the present study (Table 2). Table 3 shows comparative inter-group analysis regarding plaque and gingival indices.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Periodontally Healthy</th>
<th>Gingivitis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>73</td>
<td>49</td>
<td>122</td>
</tr>
<tr>
<td>Female</td>
<td>72</td>
<td>60</td>
<td>132</td>
</tr>
<tr>
<td>Total</td>
<td>145</td>
<td>109</td>
<td>254</td>
</tr>
</tbody>
</table>
Initially, intra-group analysis of periodontally healthy children showed *P. gingivalis* as the most frequent pathogen in intra-sulci samples, and *T. forsythia* and *A. actinomycetemcomitans* (Figure 1) were the less detected species in tongue samples. *P. gingivalis* was the most frequent pathogen in both types of sampled sites (Figure 2) among gingivitis children. Table 4 shows in details all frequency and comparative inter-group statistical analysis (periodontally healthy vs. gingivitis). *C. rectus* was more frequent in the sulci of healthy than in gingivitis children. Frequency of *P. gingivalis* was higher in gingivitis children in comparison to healthy children in both sulci and tongue samples. Table 4 also shows comparative data among sampling site (intra-sulci vs. extra-sulci vs. combined sites).

**Figure 1:** Frequency of periodontal pathogens in the healthy group. Mean values of each bacterial species were compared according to the oral site of sampling, i.e, intra-sulci, extra-sulci or both. **S:** Intra-sulci samples; **C:** Extra-sulci samples; **S+C:** Combined frequency of intra- and extra-sulci samples; ‡ statistically significant difference p <0.05; * statistically significant difference p <0.01; Chi-square test.

**Figure 2:** Frequency of periodontal pathogens in the gingivitis group. Mean values of each bacterial species were compared according to the oral site of sampling, i.e, intra-sulci, extra-sulci or both. **S:** Intra-sulci samples; **C:** Extra-sulci samples; **S+C:** Combined frequency of intra- and extra-sulci samples; ‡ statistically significant difference p <0.05; * statistically significant difference p <0.01; Chi-square test.
Discussion

Only after caries control, gingival inflammation in children received scientific attention. Also, the perception that colonization by periodontal pathogenic species occurs earlier in life [20] contributed for the interest in microbial investigation of this age group.

Although studies have been carried out to determine the prevalence of periodontal pathogens related to periodontal clinical status of children, due to the applied methodology there are many aspects that still need to be clarified [12,13,16,22-27]. In Brazil, a similar variable methodological pattern among studies could also be observed [15,28-30]. In a previous study our group [20] sampled 33 periodontally healthy children also between 6 and 12 years of age and compared among different age groups frequencies of the same bacterial species searched in the present study. On the other hand, the present cross-sectional study was designed aiming at comparing bacterial frequencies between health and gingivitis status within the same age group (6-12 years) group. It is also important to emphasize that the present study investigated a higher number of children in comparison to the one published in 2008. Now, 145 periodontally healthy children and 109 gingivitis children were investigated.

There is a lack of agreement whether gender is related to periodontal disease in children or not. According to López et al. [31] girls less than 12 years of age had a higher risk for periodontal disease in comparison to boys. On the contrary, Cortellazzi et al. [32] and Chambrone et al. [30] observed a higher prevalence of gingivitis among boys. In the present study there was no significant difference between genders regarding gingivitis occurrence (45.45% in girls and 40.16% in boys).

Our intra-group analysis revealed P. gingivalis as the most frequent species in the sulci of both healthy and diseased children. This bacterium was also one of the most frequent in tongue samples. In the gingivitis group P. gingivalis was alone the most prevalent, while in the healthy group both C. rectus and P. gingivalis shared the highest prevalence level.

Periodontal diseases represent a polymicrobial infection where microorganisms display extensive interactions: (i) competition for bacterial nutrients, (ii) synergistic interaction, (iii) antagonism when one resident inhibits the growth of another, (iv) neutralization of a bacterial nutrients, (ii) synergistic interaction, (iii) antagonism when one resident inhibits the growth of another, (iv) neutralization of a bacterial nutrient and (v) interference in the growth-dependent signaling mechanisms [33]. P. gingivalis often coexists with other periodontopathic bacteria such as P. intermedia, Fusobacterium nucleatum, T. forsythia and Treponema denticola [14,34] contributing to the higher number of interactive relations observed in periodontal biofilm.

Presence of key pathogens such as P. gingivalis, A. actinomyctecomcomitans and T. forsythia in samples of children without gingivitis confirms that biofilm is one of the factors related to disease development and that these species are members of human indigenous microbiota. For instance, our group detected in a previous study the presence of T. forsythia in newborns and babies, from 0 to 4 months of life [8]. However, considering the factor time our data also suggest the need for monitoring this population because there is no guarantee of keeping the healthy status until adult life.

Gafan et al. [13] found higher levels of T. forsythia in healthy children and Riep et al. [35] reported that P. gingivalis, P. intermedia and T. forsythia could even be detected in subjects resistant against periodontitis. In addition, an increase in pathogenic species overtime was suggested by Papapanou et al. [26]. In this context, this increase could overlap immune tolerance level anytime leading to the loss of opportunity for primary preventive measures. In older subjects (14 to 17 years), P. gingivalis showed a positive correlation with gingival index, bleeding index and probing depth [24,27].

Again, besides the presence of these bacteria there are other factors related to the development of periodontal diseases. Especially in the studied age many risk factors will change during life, impacting the overall risk for periodontal diseases.

It can be concluded that P. gingivalis was highly frequent and that C. rectus was more prevalent in healthy children. At this age group clinical status was not always directly related to presence of the searched pathogens.

Conclusion

Evolving shear stress in vessels smaller than 150 microns causes stimulated reshaping oxygen carriers. As a consequence of these changes, the liquid phase is moved by the pressure gradient from the capillary lumen into the erythrocyte. The hematocrit and the blood viscosity in the vessel are reduced. These transformations are reversible. When the erythrocyte leaves capillary, the shear deformations are reduced, cell shape is restored and the water re-enters inside. Using labeled media and fluorescent dyes, as well as experiments with cooking buffers on heavy water and subsequent stress by passing the erythrocyte suspension through a Millipore filters or by syringe hopefully confirms our conclusion.

References


