STUDYING SUBGINGIVAL MICROORGANISMS IN CHILDREN WITH GINGIVITIS IN PUBERTY

Nadezhda Mitova¹, Maya Rashkova¹, Christina Popova²
1) Department of Pediatric Dentistry, Faculty Dental Medicine, Medical University – Sofia, Bulgaria.
2) Department of Periodontology, Faculty Dental Medicine, Medical University – Sofia, Bulgaria.

ABSTRACT

Introduction: Gingival inflammations relating to an increase in the quantity of dental plaque are most frequently seen in children and young adults. Their spread and severity increase with age and reach their peak during puberty.

Aim: To study the main microorganisms of the subgingival microflora in children in puberty (10 – 14 years of age).

Material and methods: 60 children aged 10-14 years subjected to monitoring- 30 without gingivitis (up to 25% PBI) and good oral hygiene and 30 children with plaque-induced gingivitis (over 50% PBI). A PCR – Real-Time method was used for identifying the main subgingival microorganisms and determining their quantities. Samples were taken with paper pins from the gingival sulcus of six teeth – three molars, two canines and one incisor (16, 13, 11, 26, 36, 43). Nine control strains were studied (a comprehensive sample). Samples were sent for research in standardized containers.

Results: The results of this study show that the total quantity of subgingival microorganisms increases in the case of worsening oral hygiene and an increase in the quantity of dental biofilm. Six of the nine subgingival microorganisms tested were encountered in all children between the ages of 10 and 14. The remaining three species of microorganisms were isolated only from the children with gingivitis.

Conclusion: During gingival inflammations, the subgingival microflora becomes more complex and from it can be isolated microorganisms from the red complex (P. gingivalis, T. denticola, T. forsythia).

Keywords: subgingival microorganisms, PCR-Real Time, periodontal diseases

INTRODUCTION

Gingival inflammations relating to an increase in the quantity of dental plaque are most frequently seen in children and young adults. Their spread and severity increase with age and reach their peak during puberty [1]. Multiple studies show that changes in hormonal levels are related to an increase in the spread and severity of gingival illnesses [2, 3].
MATERIALS AND METHODS

Subjects of the study were 60 children between the ages of 10 and 14, who did not suffer from any systemic diseases and had not had any antibiotic intake three months prior. The children were distributed into two groups:
- 30 children without gingivitis (up to 25% Papilla Bleeding index (PBI) Saxer & Mulheman (spread)) and with good oral hygiene - average OHI-Green Vermillion simplified = 0.56;
- 30 children with plaque-induced gingivitis (over 50% Papilla Bleeding index (PBI) Saxer & Mulheman (spread)) – average OHI-Green Vermillion simplified = 2.15

A PCR – Real-Time method was used for identifying the main subgingival microorganisms and determining their quantities. Samples were taken with paper pins from the gingival sulcus of six teeth – three molars, two canines and one incisor (16, 13, 11, 26, 36, 43). The samples were taken in the morning – around 9 – 10 o’clock, at least half an hour after the teeth had been brushed and at least an hour after eating, after which they were sent in standardized containers for testing.

Nine control strains were studied (a comprehensive sample), table 1

Table 1. The microorganisms tested, as grouped by Socransky

<table>
<thead>
<tr>
<th>Microorganisms Tested</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinomyces actinomycetemcomitans</td>
<td>Purple complex</td>
</tr>
<tr>
<td>Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia</td>
<td>Red complex</td>
</tr>
<tr>
<td>Prevotella intermedia, Peptostreptococcus (micromonas) micros, Fusobacterium nucleatum, Eubacterium nodatum,</td>
<td>Orange complex</td>
</tr>
<tr>
<td>Capnocytophaga gingivalis</td>
<td>Green complex</td>
</tr>
</tbody>
</table>

Unlike the standard PCR, Real-Time-PCR allows not only for the endpoint for the reaction to be determined but also for the quantity of the PCR-product. Real-Time-PCR always employs fluorescence. Increasing the fluorescent signal is proportional to the quantity of DNA multiplied. Calculations at the end of the process were carried out with the help of a software program.

RESULTS

1. Description of the total quantity of microorganisms tested

1.1. Total quantity of the subgingival microorganisms

The average quantities of microorganisms in healthy children are 3.7x10^7, while the quantities of microorganisms isolated from the children with gingivitis are plausibly higher – 9.1x10^7 (t=-0.525, P<0.05).

The total quantity of subgingival microorganisms increases with the development of gingival inflammation in children during the period of sexual maturation.

1.2 Correlation between the quantity of dental plaque (OHI – GV) and the quantity of the microorganisms tested.

The correlation between the quantity of dental plaque and the quantity of isolated subgingival microorganisms from all subjects was analyzed in this study. The results are presented in the following tables and diagrams.

Table 2. Total quantity of microorganisms in children with and without plaque induced gingivitis

<table>
<thead>
<tr>
<th></th>
<th>healthy</th>
<th>gingivitis</th>
<th>Ind T-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean ± SD</td>
<td>N</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>3.7x10^7 ± 5.6x10^7</td>
<td>30</td>
</tr>
</tbody>
</table>

Table 3. Total quantity of microorganisms (MO), grouped according to the OHI of the children tested

<table>
<thead>
<tr>
<th></th>
<th>Quantity of MO with OHI≤1</th>
<th>Quantity of MO with 1&gt;OHI&lt;2</th>
<th>Quantity of MO with OHI≥2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean ± SD</td>
<td>N</td>
</tr>
<tr>
<td>total</td>
<td>23</td>
<td>3.5x10^7 ± 5.7x10^7</td>
<td>18</td>
</tr>
<tr>
<td>IndT-test total</td>
<td>t_{1,2}=-1.539</td>
<td>p=0.132</td>
<td>t_{1,2}=-2.212</td>
</tr>
</tbody>
</table>

The data presented in the table shows that with an increase in the OHI values, an increase in the total quantity of isolated subgingival microorganisms is also observed, with a plausible difference being present between the groups with the least and most amount of plaque (p<0.05). Therefore in cases of bad oral hygiene, a plausible increase in the total microbial load of the subgingival microorganisms tested is observed.

The table shows that *Capnocytophaga gingivalis* (green complex) was isolated from all children – 100%, followed by *F. nucleatum* (orange complex) – 81.7%.

*Capnocytophaga* is a commensal species from the green complex, as grouped by Socransky, and is considered an opportunistic pathogen. It participates in various types of infections, including periodontal infections, the severity of which depends on the patient’s immune status.

*F. nucleatum* is one of the secondary colonizers, which are incorporated into the biofilm and is considered an intermediary colonizer and a mediator between the supra and subgingival microflora of mostly periodontal pathogen strains.

*Peptostreptococcus micros* – 35.0%, which is also a part of the orange complex was isolated in 1/3 of the cases. It belongs to the gram-positive cocci, is anaerobic and is part of the commensal subgingival microflora.

*Prevotella intermedia* (P.i.) – 18.3% is the next most frequently encountered microorganisms from the orange complex, as grouped by Socransky. It is part of the black-pigmented microorganisms of the *Bacteroides* group. It is a gram-negative rod, strictly anaerobic and a commensal that is strongly associated with the development of periodontal illnesses.

*Eubacterium nodatum* (E.n.) is a gram-positive rod, anaerobic, a commensal, with a moderate association towards the development of periodontal illnesses. It was isolated from a small number of subjects in this study.

The results of this study show that the most frequently isolated representatives of the red complex are *T. denticola* – 36.7%, followed by *T. forsythia* – 20% and *P. gingivalis* – 13.3%. *T. forsythia* and *T. denticola* were encountered in isolated cases among the children with gingivitis.

*A. actinomycetemcomitans* was encountered in 11.7% of the analyzed samples. *Aggregatibacter actinomycetemcomitans* (a G-negative microaerophilic microorganism) is considered a main microbial factor in aggressive periodontitis, usually with early-onset and combined with various defects in the immune response, owed to vari-

### Table 4. Subgingival microflora of the children tested – total.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>N</th>
<th>Isolated</th>
<th>t</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. actinomycetemcomitans</em> (1)</td>
<td>7</td>
<td>11.7±4.14</td>
<td>t1,3 = 0.28, t1,5 = 3.06</td>
</tr>
<tr>
<td><em>P. gingivalis</em> (2)</td>
<td>8</td>
<td>13.3±4.38</td>
<td>t1,4 = 3.34, t2,4 = 0.98</td>
</tr>
<tr>
<td><em>T. denticola</em> (3)</td>
<td>22</td>
<td>36.7±6.22</td>
<td>t3,5 = 15.49, t3,7 = 5.64</td>
</tr>
<tr>
<td><em>T. forsythia</em> (4)</td>
<td>12</td>
<td>20.0±5.16</td>
<td>t4,6 = 15.49, t4,9 = 15.49</td>
</tr>
<tr>
<td><em>P. intermedia</em> (5)</td>
<td>11</td>
<td>18.3±5.00</td>
<td>t5,8 = 14.23, t5,9 = 14.23</td>
</tr>
<tr>
<td><em>P. (micromonas) micros</em> (6)</td>
<td>21</td>
<td>35.0±6.16</td>
<td>t6,9 = 10.56, t6,10 = 10.56</td>
</tr>
<tr>
<td><em>F. nucleatum</em> (7)</td>
<td>49</td>
<td>81.7±5.00</td>
<td>t7,9 = 14.23, t7,10 = 14.23</td>
</tr>
<tr>
<td><em>E. nodatum</em> (8)</td>
<td>2</td>
<td>3.3±2.32</td>
<td>t8,9 = 19.95, t8,10 = 19.95</td>
</tr>
<tr>
<td><em>C. gingivalis</em> (9)</td>
<td>60</td>
<td>100±0</td>
<td>t9,10 = 41.71, t9,11 = 41.71</td>
</tr>
</tbody>
</table>
ous gene polymorphisms or defects in phagocytosis [4, 5, 9].

2.2. Comparison of the frequency of the subgingival microorganisms tested in children with and without gingivitis

It is noticeable that the tree of the microorganisms tested was isolated only from children with gingivitis: A. actinomycetemcomitans, P. gingivalis, and from only two children – E. nodatum.

From both groups, T. denticola and T. forsythia (red complex) were isolated, with this percentage being much higher in children with gingivitis (P<0.05).

As far as orange complex microorganisms, F. nucleatum is relatively evenly distributed between the group with gingivitis and the healthy group, while P. intermedia and P. micros were plausibly more frequently encountered in the children with plaque-associated gingivitis.

C. gingivalis (green complex), which was encountered in all the children tested, was also evenly distributed between the healthy group and the group with gingivitis.

**DISCUSSION**

The results of this study show that the total quantity of subgingival microorganisms increases in the case of worsening oral hygiene and an increase in the quantity of dental biofilm. From the point of view of species diversity among the subgingival microorganisms, however, this study presents a new very interesting information regarding the microbial ecology of the subgingival biofilm during the period immediately after the eruption of permanent teeth.

The results show that six of the nine subgingival microorganisms tested were encountered in all children between the ages of 10 and 14. The remaining three species of microorganisms were isolated only from the children with gingivitis, them being A. actinomycetemcomitans and P. gingivalis, each of which was isolated in 12% of the children and E. nodatum, which was encountered in an insignificantly small percentage of children.

Microorganisms with weaker pathogenicity (orange complex) and microorganisms necessary for the initiation of the processes of coaggregation, which are important for the development of the subgingival biofilm (F. nucleatum, C. gingivalis) were encountered in the healthy group with low quantities of plaque. As a gingival inflammation develops, the subgingival microflora becomes more complex. From it, red complex microorganisms can be isolated (P. gingivalis, T. denticola, T. forsythia). In such cases, the frequency of microorganisms from the orange complex (P. intermedia, P. micros) also increases. *Prevotella intermedia* participates in periodontal infections, including gingivitis and periodontitis, and is often encountered in cases of acute necrotizing ulcerative gingivitis. According to data found in scientific literature,
an increase in the quantity of \textit{P. intermedia} is observed during puberty, especially in boys [7, 9].

The presence of \textit{T. forsythia} and \textit{T. denticola} even in minimal quantities is considered a risk factor for the initiation of periodontal disease in children in puberty [7, 10, 11].

According to a recent study by Ning-Yan, \textit{P. gingivalis}, \textit{P. intermedia} and \textit{T. forsythensis} are found in higher levels in samples, taken from children with periodontal diseases, when compared to the samples taken from healthy children. All species of microorganisms are found in higher levels in the groups with more severe periodontal pathology. This presupposes a possible connection between the quantity of \textit{P. gingivalis}, \textit{P. intermedia}, \textit{T. forsythensis} and \textit{F. nucleatum}, and gingival inflammations [8]. A point with is confirmed by the authors by the observation of a significant correlation between the levels of the aforementioned microorganisms and the clinical indexes (PI, GI, SBI and PD) [8, 12, 13].

Many authors consider that the initiation and progression of periodontal diseases are directly linked to the colonization of microorganisms, including Aggregatibacter actinomycetemcomitans, as well as microorganisms from the red complex, as grouped by Socransky [12, 14]. This study confirms that conclusion.

Kulekci has identified microorganisms associated with periodontal destruction in small children and adolescents [11]. According to Tanner and Papaioannou, not only the dental biofilm can be a reservoir for bacteria, associated with periodontal illnesses, but the tongue can also be colonized by these species, even in children as small as six months old. The authors of the study used a PCR-test for identification [10, 15].

Another study showed similar results. A difference in the quantity of \textit{P. gingivalis}, \textit{P. intermedia}, \textit{T. forsythensis} and \textit{F. nucleatum} in the subgingival plaque of developing children with a varying periodontal status was discovered. The quantity of these periodontal pathogens increases along with the increase in the severity of the inflammation. The same tendency is observed in cases of severe gingivitis and periodontitis [16, 17, 18].

Armitage reports that the suspected periodontal pathogens are frequently also found in healthy individuals, not suffering from periodontal illnesses, where they are however, in limited quantities [19]. This result shows that most periodontal pathogens are often present in the subgingival plaque of children, regardless of their periodontal status or the presence or absence of inflammation, but the quantity in which they are present varies [20, 21].

**CONCLUSIONS:**

1. As the quantity of plaque increases, so does also the total quantity of the isolated subgingival microorganisms;

2. Microorganisms with weaker pathogenicity (\textit{P. intermedia}, \textit{P. micros} – orange complex) and microorganisms necessary for the initiation of the processes of coaggregation (\textit{F. nucleatum}, \textit{C. gingivalis}) are encountered in children with lower quantities of plaque;

3. During gingival inflammations, the subgingival microflora becomes more complex and from it can be isolated microorganisms from the red complex (\textit{P.gingivalis}, \textit{T.denticola}, \textit{T.forsthyia}). Also, the frequency of microorganisms from the orange complex increases.

This publication is the result of a study under a project, financed by the Council of Medical Science under MU - Sofia.

**REFERENCES:**

1. Rashkova M. [Periodontal diseases in children and adolescents] [monograph]. Sofia: Direct Services; 2016. [in Bulgarian]


8. Yang NY, Zhang Q, Li JL, Yang SH, Shi Q. Progression of periodontal inflammation in adolescents is associated with increased number of Porphyrmonas gingivalis, Prevotella intermedia, Tannerella forsythians, and Fusobacterium nucleatum. \textit{Int J Paediatr Dent}. 2014 May;24(3):226-33. [PubMed] [Crossref]


Address for correspondence
Nadezhda Georgieva Mitova
Department of Pediatric Dentistry, Faculty of dental medicine, Medical University – Sofia,
1, Georgi Sofiisky str., Sofia, Bulgaria.
Phone: 00359 029533475, 00359 886216886,
E-mail: nadia_bm@abv.bg

Please cite this article as: Mitova N, Rashkova M, Popova Ch. Studying subgingival microorganisms in children with gingivitis in puberty. J of IMAB. 2019 Oct-Dec;25(4):2822-2827. DOI: https://doi.org/10.5272/jimab.2019254.2822

Received: 28/05/2019; Published online: 09/12/2019