https://doi.org/10.5272/jimab.2019254.2822

Journal of IMAB - *Annual Proceeding (Scientific Papers)*. 2019 Oct-Dec;25(4)

Journal of IMAB
ISSN: 1312-773X
https://www.journal-imab-bg.org

Original article

STUDYING SUBGINGIVAL MICROORGANISMS IN CHILDREN WITH GINGIVITIS IN PUBERTY

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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].

Studies show that after the onset of puberty a substantial change in the oral microflora occurs – an increase in cocci levels (*Streptococcus* and *Veillonella)*, rod-shaped bacteria (*Actinomyces, Prevotela, Fusobacterium)*, spirochaetes *(Capnocytophaga, Eikenella*and *Veillonella)*. Gingivitis is related to higher and/or predominant levels of *Eikenella, B. intermedius, Fusobacterium* and spirochetes, while healthy children exhibit higher levels of *Actinomyces viscous.* Thus it can be determined that the presence or absence of an illness is determined by the microbial changes, observed in both groups [4, 5, 6].

There exists data that the heightened levels and proportions of black-pigmented *Bacteroides* observed before, during, and shortly after puberty correlate with the levels of gingival inflammation. According to some authors, and contrary to expectations, no correlation between the heightened levels of steroid hormones and the heightened proportions of these microorganisms *(Bacteroides)* is observed.Meaning that the presence of gingival inflammation, and not heightened sex hormone levels, is what correlates with *Bacteroides* levels. The contradictory nature of the results requires that this problem be studied more in-depth [6, 7, 8].

Studying the composition of the subgingival microflora immediately after the eruption of permanent teeth during puberty will give basis for an in-depth analysis of the periodontal status, at the time of the highest risk age for periodontal pathology of adolescence, and to provide a description of the periodontal pathology during said age, with the goal of developing a preventive approach and specific treatment.

Aim

To study the main microorganisms of the subgingival microflora in children during the process of sexual maturation (10 – 14 years of age).

OBJECTIVES

1. To provide a description of the total quantity of the subgingival microorganisms studied.

2. To provide a description of the types of isolated MO from the subgingival microflora of the children tested.

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 Hygiene Index (HI) = 32.2.

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

Actinomyces actinomycetemcomitans,	Purple complex
Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia,	Red complex
Prevotela intermedia, Peptostreptococcus (micromonas) micros, Fusobacterium nuleatum, Eubacterium nodatum,	Orange complex
Capnocytophaga gingivalis.	Green complex

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*

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

	healthy		gingivitis		Ind T-test
	N	Mean ± SD	N	Mean ± SD	
Total	30	$3.7x10^7 \pm 5.6x10^7$	30	$9.1x10^7 \pm 1.0x10^8$	$t_{1,2}$=-2.525 p=0.014

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

	Quantity of MO with OHI≤1		Quantity of MO with 1>OHI<2		Quantity of MO with OHI≥2	
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
total	23	$3.5x10^7 \pm 5.7x10^7$	18	$6.8x10^7 \pm 6.8x10^7$	19	$9.3x10^7 \pm 1.1x10^7$
IndT-test total quantity	$t_{1,2}$=-1.539 p=0.132		$t_{1,3}$=-2.213 p=0.033		$t_{2,3}$=-0.758 p=0.454	

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.

2. Description the types of microorganisms isolated from the subgingival microflora of the children tested

2.1. Description of the species of microorganisms tested in total in all children.

An identification of the main subgingival microorganisms was carried out in all children in the study (a comprehensive sample of nine control strains), the average quantities of these microorganisms were also determined. The results are presented in the following tables.

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

Microorganisms	N	Isolated %±sp	
A.actinomycetemcomitans (1)	7	11.7±4.14	$t_{1,2}$= 0.28 $t_{2,3}$= 3.06
P.gingivalis (2)	8	13.3±4.38	$t_{3,5}$= 2.30 $t_{4,8}$= 2.94
T.denticola (3)	22	36.7±6.22	$t_{6,9}$= 10.56 $t_{1,3}$= 3.34
T.forsythia (4)	12	20.0±5.16	$t_{2,4}$= 0.98 $t_{3,6}$= 0.19
P.intermedia (5)	11	18.3±5.00	$t_{4,9}$= 15.49 $t_{7,8}$= 14.23
P.(micromonas)micros (6)	21	35.0±6.16	$t_{1,4}$= 1.26 $t_{2,5}$= 0.75
F.nucleatum (7)	49	81.7±5.00	$t_{3,7}$= 5.64 $t_{5,6}$= 2.10
E.nodatum (8)	2	3.3±2.32	$t_{7,9}$= 3.67 $t_{1,5}$= 1.03
C.gingivalis (9)	60	100±0	$t_{2,6}$= 2.87 $t_{3,8}$= 5.02
			$t_{5,7}$= 8.96 $t_{8,9}$= 41.71
			$t_{1,6}$= 3.14 $t_{2,7}$= 10.28
			$t_{3,9}$= 10.18 $t_{5,8}$= 2.72
			$t_{1,7}$= 10.78 $t_{2,8}$= 2.01
			$t_{4,5}$= 0.23 $t_{5,9}$= 16.35
			$t_{1,8}$= 1.76 $t_{2,9}$= 19.95
			$t_{4,6}$= 1.87 $t_{6,7}$= 5.89
			$t_{1,9}$= 21.31 $t_{3,4}$= 2.06
			$t_{4,7}$= 8.58 $t_{6,8}$= 4.81

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-

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*

Table 5. Frequency of the subgingival microorganisms studied according to the level of gingival inflammation

Microorganisms	Healthy		Gingivitis		Total		Chi-square Test
	N	%	N	%	N	%	
A.actinomycetemcomitans	0	0	7	100	7	100	χ =7.925 p=0.005
P.gingivalis	0	0	8	100	8	100	χ =9.231 p=0.002
T.denticola	3	13.6	19	86.4	22	100	χ =18.373 p=0.000
T.forsythia	1	8.3	11	91.7	12	100	χ =10.417 p=0.001
P.intermedia (5)	2	18.2	9	81.8	11	100	χ =5.455 p=0.020
P.(micromonas)micros (6)	6	28.6	15	71.4	21	100	χ =5.934 p=0.015
F.nucleatum (7)	21	42.9	28	57.1	49	100	χ =5.455 p=0.020
E.nodatum (8)	0	0	2	100	2	100	χ =2.069 p=0.150
C.gingivalis (9)	30	50	30	50	60	100	χ = p=

It is noticeable that the tree of the microorganisms tested was isolated only from children with gingivitis: *A. actinomycetemcomitans, P. gingivalis.*andfrom 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 *P. intermedia* is observed during puberty, especially in boys [7, 9].

The presence of *T. forsythia* and *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, *P. gingivalis, P. intermedia*and *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 *P. gingivalis, P. intermedia, T. forsythensis*and *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 *P. gingivalis, P. intermedia, T. forsythensis* and *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].

Armitagereports 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 (*P. intermedia, P. micros* – orange complex) and microorganisms necessary for the initiation of the processes of coaggregation (*F. nucleatum, 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 (*P.gingivalis, T.denticola, T.forsythia).* 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.

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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

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