



SPECIES CHARACTERISTICS OF MICROBIAL ASSOCIATIONS IN THE COURSE OF TREATMENT OF 10 TO 14-YEAR-OLD CHILDREN WITH PLAQUE-INDUCED GINGIVITIS

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SUMMARY

Aim: A valuation of the species characteristics of subgingival biofilm microbial associations during treatment of children with plaque-induced gingivitis aged 10-14 years.

Materials and methods: The subject of the study were 34 children with plaque-induced gingivitis. Three samples of subgingival biofilm are taken per patient: 1st sample - at the first visit; 2nd sample - two weeks later; 3rd the sample - one month later. Five teeth per child are selected from which a sample is taken for PCR testing for 9 control strains of subgingival microorganisms (*A. actinomycetemcomitans*, *P. gingivalis*, *T. denticola*, *T. forsythia*, *P. intermedia*, *P. micros*, *F. nucleatum*, *E. nodatum*, *C. gingivalis*).

Results: *C. gingivalis* (green complex) is isolated in all examined children before the start of treatment and is indispensably observed in microbial associations with *P. intermedia* (orange complex). In other cases, associations with 7-8 microorganisms are observed and in a more complex composition. One month after the start of treatment, no periodontopathogens or only *C. gingivallis* as a single microorganism was isolated in more than 55% of the samples examined. Microbial associations are predominantly 2 to 4 microorganisms.

Keywords: microbial associations, gingivitis in children, periodontopathogens in gingivitis, Socransky complexes,

INTRODUCTION

Microorganisms are considered a key etiological factor in the initiation of gingival inflammation. Modern scientific studies link the pathogenesis of periodontal diseases to the imbalance in the subgingival microflora, which is due to an increased amount of certain periodontopathogens.

Dental biofilm is defined as a highly organized microbial community composed of cells attached to the tooth surface or embedded in an extracellular matrix. The microorganisms forming the dental biofilm change their phenotype in response to the change in living conditions in the community and, as a result, acquire the ability for faster growth, adaptation and potentiated pathogenic potential [1].

Scientists have established experimentally that specific interactions exist between dental biofilm microorganisms and host gingival tissues [2]. The amount of dental biofilm required to initiate gingival inflammation and its relationship to the rate of progression vary greatly between individuals [3].

Through microscopic studies, scientists have established that there is a strictly defined order in the structure of the dental biofilm, in which certain microorganisms dominate or are in close association with other microorganisms united in microbial associations [4, 5].

Socransky established 5 closely related groups of microorganisms based on their simultaneous presence in a certain niche. Yellow, green and purple complex, or the so-called initial colonizers, preceded the settlement of Gr (-) MO by the red and orange complex [6].

In healthy children, the subgingival microflora is dominated by less pathogenic microorganisms than the purple and green complex, according to Socransky, while in children with gingivitis, a tendency for quantitative and species diversity of the subgingival biofilm, as well as complex relationships between individual species, is gradually observed [7, 8, 9]. This suggests that the tran-

sition from health to disease (gingivitis) follows the principles of characteristic primary ecological settlement, which determines our interest in the species-specific characterization of microbial associations in children with established gingivitis.

MATERIALS AND METHODS

The subject of the study were 34 children with generalized plaque-induced gingivitis. Clinical periodontal examination was performed by levels, the first three included assessment of systemic and behavioral risk factors, assessment of the oral environment, teeth and gingiva. Assessment of the gingival sulcus was performed using an electronic periodontal probe PA ON (Orange dental).

The plaque-induced gingivitis treatment protocol included a total of 5 visits for each child, providing motivation and training in individual plaque control, standard mechanical periodontal therapy, corrective therapy and control.

To assess the species characterization of microbial associations, three subgingival biofilm samples are taken per patient: 1st sample - at the first visit; 2nd sample - two weeks later; 3rd the sample - one month later. Five teeth per child are selected with the most severe gingival pathology (highest probing depth, bleeding on probing index over 50 % per tooth, sulcus bleeding index over 2), from which a sample is taken for PCR testing.

A Real Time PCR method was used to identify major subgingival microorganisms - 9 control strains of subgingival microorganisms (*A. actinomycetemcomitans*, *P. gingivalis*, *T. denticola*, *T. forsythia*, *P. intermedia*, *P. micros*, *F. nucleatum*, *E. nodatum*, *C. gingivalis*) grouped into 4 Socransky complexes.

RESULTS

The following table presents the microbial associations according to the type of microorganisms investigated in the initial examination of the children (table 1).

Table 1. Microbial associations before treatment

	Microbial associations			total	
		N	%	N	%
1 MO	<i>C. gingivallis</i>	4	11.7	4	11.7
2 MO	<i>C. gingivallis</i> + <i>P. intermedia</i>	2	5.8	4	11.7
	<i>C. gingivallis</i> + <i>P. micros</i>	2	5.8		
3 MO	<i>C. gingivallis</i> + <i>F. nucleatum</i> + <i>P. micros</i>	4	8,8	6	17.6
	<i>C. gingivallis</i> + <i>P. intermedia</i> + <i>E. nodatum</i>	2	8,8		
4 MO	<i>C. gingivallis</i> + <i>P. intermedia</i> + <i>T. denticola</i> + <i>T. forsythia</i>	2	5,8	2	5.8
5 MO	<i>C. gingivallis</i> + <i>P. gingivalis</i> + <i>P. intermedia</i> + <i>P. micros</i> + <i>E. nodatum</i>	2	5,8	4	11.7
	<i>C. gingivallis</i> + <i>P. micros</i> + <i>P. intermedia</i> + <i>T. denticola</i> + <i>T. forsythia</i>	2	5,8		
6 MO	<i>C. gingivallis</i> + <i>E. nodatum</i> + <i>P. micros</i> + <i>P. intermedia</i> + <i>T. denticola</i> + <i>T. forsythia</i>	2	5,8	5	14.7
	<i>C. gingivallis</i> + <i>F. nucleatum</i> + <i>P. micros</i> + <i>P. intermedia</i> + <i>T. denticola</i> + <i>T. forsythia</i>	3	8,8		
7 MO	<i>C. gingivallis</i> + <i>F. nucleatum</i> + <i>P. micros</i> + <i>P. intermedia</i> + <i>T. denticola</i> + <i>T. forsythia</i> + <i>P. gingivalis</i>	2	5,8	7	20,5
	<i>C. gingivallis</i> + <i>P. micros</i> + <i>P. intermedia</i> + <i>T. denticola</i> + <i>T. forsythia</i> + <i>P. gingivalis</i> + <i>A. actinomycetemcomitans</i>	2	5,8		
	<i>C. gingivallis</i> + <i>P. micros</i> + <i>T. denticola</i> + <i>T. forsythia</i> + <i>P. gingivalis</i> + <i>P. intermedia</i> + <i>E. nodatum</i>	3	8,7		
8 MO	<i>C. gingivallis</i> + <i>P. micros</i> + <i>P. intermedia</i> + <i>T. denticola</i> + <i>T. forsythia</i> + <i>P. gingivalis</i> + <i>E. nodatum</i> + <i>A. actinomycetemcomitans</i>	2	5.8	2	5.8

C. gingivalis (green complex) is isolated in all examined children before the start of treatment and is indispensably observed in microbial associations with *P. intermedia* (orange complex). Microorganisms of the red complex are found in almost all of the shown combinations where there are at least 4 species involved. *P. gingivalis* occurs mainly in microbial associations of at

least 7 microorganisms. *A. actinomycetemcomitans* occurs in four of the combinations, these mainly being the more numerous microbial associations (7-8 microorganisms).

The following table presents the microbial associations according to the type of microorganisms tested two weeks after the start of treatment of plaque-induced gingivitis (table 2).

Table 2. Microbial associations after two weeks

	Microbial associations			Total	
		N	%	N	%
without MO		3	8.8	3	8.8
1 MO	<i>C. gingivallis</i>	16	47	16	47
2 MO	<i>C. gingivallis</i> + <i>P. micros</i>	2	5.8	2	5.8
3 MO	<i>C. gingivallis</i> + <i>F. nucleatum</i> + <i>P. intermedia</i>	2	5,8	9	26.4
	<i>C. gingivallis</i> + <i>T. forsythia</i> + <i>T. denticola</i>	2	5,8		
	<i>P. micros</i> + <i>T. denticola</i> + <i>A. actinomycetemcomitans</i>	2	5,8		
	<i>P. micros</i> + <i>T. denticola</i> + <i>P. intermedia</i>	3	8,7		
4 MO	<i>C. gingivallis</i> + <i>P. intermedia</i> + <i>T. denticola</i> + <i>P. gingivalis</i>	2	5,8	2	5.8
6 MO	<i>T. denticola</i> + <i>E. nodatum</i> + <i>P. micros</i> + <i>P. intermedia</i> + <i>T. forsythia</i> + <i>P. gingivalis</i>	2	5,8	2	5.8

Two weeks after the start of treatment, none of the subgingival periodontopathogens were isolated in 8% of the subjects. It was also observed that microbial associations of 7 and 8 microorganisms were not registered with any of the examined persons. In cases where microbial associations were isolated, again, *C. gingivalis* (green complex) was present in all samples alone or in combination, usually with *P. intermedia*. In more complex combinations,

microorganisms from the red complex also take part. *A. actinomycetemcomitans* was not isolated in any of the samples examined.

The following table presents the microbial associations according to the type of microorganisms tested one month after the start of treatment of plaque-induced gingivitis (table 3).

Table 3. Microbial associations after one month

	Microbial associations			Total	
		N	%	N	%
without MO		3	8.8	3	8.8
1 MO	<i>C. gingivallis</i>	16	47	16	47
2 MO	<i>C. gingivallis</i> + <i>P. micros</i>	2	5.8	2	5.8
3 MO	<i>C. gingivallis</i> + <i>F. nucleatum</i> + <i>P. intermedia</i>	2	5,8	9	26.4
	<i>C. gingivallis</i> + <i>T. forsythia</i> + <i>T. denticola</i>	2	5,8		
	<i>P. micros</i> + <i>T. denticola</i> + <i>A. actinomycetemcomitans</i>	2	5,8		
	<i>P. micros</i> + <i>T. denticola</i> + <i>P. intermedia</i>	3	8,7		
4 MO	<i>C. gingivallis</i> + <i>P. intermedia</i> + <i>T. denticola</i> + <i>P. gingivalis</i>	2	5,8	2	5.8
6 MO	<i>T. denticola</i> + <i>E. nodatum</i> + <i>P. micros</i> + <i>P. intermedia</i> + <i>T. forsythia</i> + <i>P. gingivalis</i>	2	5,8	2	5.8

One month after the start of treatment, no periodontopathogens or only *C. gingivallis* as an independent microorganism were isolated in more than 55% of the examined samples. Microbial associations are mostly 2 to 4 microorganisms, with *C. gingivallis* not necessarily present in each of the combinations, but a variety of representatives of the orange and red complex is observed. In two of the studied samples, *A. actinomycetemcomitans*

was also isolated in combination with *T. denticola* from the red complex and *P. micros* from the orange complex.

DISCUSSION

Dental biofilm is a leading etiological factor in plaque-induced gingivitis, and the study of the species characteristics of microbial association of the subgingival microflora in children with PIG, as well as assessment

of their pathogenic potential in the course of treatment, are the subject of the present study.

With the eruption of the permanent teeth, after the formation of a supragingival biofilm, the subgingival biofilm is also formed, as a reflection of its composition, both the changing conditions in the gingival sulcus and the presence of gingival inflammation [10]. These features create suitable conditions for more complex microbial associations with potentiated virulence qualities [11].

In the present study, the real-time PCR method was used for the identification of the main subgingival microorganisms in children with PIG, as well as the study of the change in the subgingival microflora during treatment.

The current study shows that the most frequently isolated periodontopathogen in children with generalized plaque-induced gingivitis is *C. gingivalis*, which occurs in 100% of the examined children. *C. gingivalis* is representative of the green complex, according to Socransky and is part of the so-called early colonizers, and probably its role in periodontal pathology is primarily related to the creation of conditions favoring the settlement of other periodontopathogens with marked virulent qualities [10]. Another reason to consider this MO as an intermediary in the construction of the subgingival microflora is that it is most often found in microbial associations with other representatives of the Socransky orange complex with pronounced coaggregation qualities (*F. nucleatum*, *P. micros*). Similar to our results, other authors established the same regularities regarding isolation of *C. gingivalis* [12,8].

We also found that a typical finding for children with generalized plaque-induced gingivitis is that microbial associations is formed from at least 7 MO with repre-

sentatives of the orange and red Socransky complexes predominate.

It is noteworthy that during the course of treatment, changes were observed in the colonization of the various species of periodontopathogens studied against the background of preservation of the total microbial load. This probably leads to a change in the subgingival ecology, which may prove to be key in the healing process in the gingiva. Similar results were obtained by a group of American authors who claim that periodontal diseases are the result of a change in the ecological relationships of the subgingival microflora [11].

The present study found that one month after the start of treatment, the relative share of *C. gingivalis* as independent MO was the highest. In 8 percent of the cases, no periodontopathogens or a microbial association of 3 MOs were isolated.

The results of the present study show that the microbial associations at the beginning of treatment of children with PIG are mainly from 6 - 8 MO, and during the course of treatment, the species diversity in the subgingival space decreases, which is most likely closely related to the healing process. We also found that in older children at 14 years of age, the subgingival ecosystem shows a clear tendency for a more complex combination of MO, which can probably be linked to the accompanying puberty changes in the child's organism, which was also found by another author's team [13].

CONCLUSION

In the course of treatment of plaque-induced gingivitis in children aged 10-14 years, the number of periodontopathogens studied decreases from 7-8 MO to 2-3 MO in microbial association.

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