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Original article

CLINICAL AND MICROBIOLOGICAL DIAGNOSIS OF PLAQUE-INDUCED GINGIVITIS IN CHILDREN AND ADOLESCENTS

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SUMMARY

Aim: Clinical assessment of periodontal status and subgingival microflora in children aged 10-14 years with plaque-induced gingivitis.

Materials and methods: The subject of the study were 34 children. The real-time PCR method was used for the identification of 9 subgingival microorganisms.

Results: The studied children showed a probing depth averaging 1.26±0.24 mm and unsatisfactory oral hygiene (FMPS - 62.76±22.94). A generalized form of gingival inflammation was found with values of bleeding sites over 52% (BoP -52.76±17.57) and mild gingival inflammation (SBI - 1.17±0.73). *C. gingivalis* was isolated in all children (100%), followed by *P. intermedia* and *P. micros* - in 70.6%. Microbial associations ranged from 2 to 8 microorganisms.

Conclusion: Children aged 10-14 years are at risk for gingival inflammation because of the pronounced pathogenic potential of the subgingival microflora.

Keywords: gingivitis in children, subgingival microflora, microbial complexes,

INTRODUCTION

Plaque-induced gingivitis is second in terms of the frequency of spreading plaque-dependent oral diseases in children and adolescents. Often these conditions are not noticed by the patients and could lead to the development of destructive inflammatory processes in the periodontal tissues [1].

The contemporary classification of periodontal diseases is linked to the emphasis on local risk factors and the inflammation in the gingiva by them in the context of the general gingival status of children. The presence of a limited margin of gingival inflammation (BoP up to 10 %) is still insufficient to diagnose gingivitis, but this condition is of interest from the point of view of the preventive method in periodontology, which is especially important in childhood [2].

The multifactorial character of periodontal diseases requires the detection not only of etiological factors but also of all risk factors, which could facilitate the development of the disease. The manifestation of the periodontal pathology as a clinical symptom is due to the complex system of interactions between the subgingival microflora, the organism's immune response and modifying factors from the environment [3,4]. Research of the subgingival biofilm in healthy children with a little amount of plaque demonstrates that microorganisms with less pathogenicity are predominant, as well as those necessary to initiate coaggregation processes [5]. The literature review lacks detailed research on the microbial composition in diagnosed plaque-induced gingivitis in children and adolescents.

For the microbiological identification of bacterial species, throughout the years, cultural methods have been mostly used to isolate species, which have low sensitivity, complex implementation technique (need for selective environments) and are time-consuming (settlement, cultivation) [6,7,8]. In contrast, the Polymerase Chain Reaction (PCR) is considered the most efficient method to identify periodontal pathogens without cultivation, based on the isolation of specific microbial DNA and the ability to identify a wide spectrum of species even when their quantities in the sample are minimal. In addition, Real Time PCR also produces a quantitative analysis of the isolated species, which makes it even more useful despite its high price [7,9].

In the puberty period, dependence between the plaque quantities, inflammation and diversity of microbial associations of microorganisms could be observed. This proves the presence of a diverse subgingival microflora with pronounced pathogen potential [10]. This determines the increased risk of periodontal pathology during this age.

Aim

The aim of the current research is to clinically assess the periodontal status and the subgingival microflora in children with plaque-induced gingivitis aged 10-14.

MATERIALS AND METHODS

The subject of the research were 34 children (aged 10-14) with plaque-induced gingivitis – instigated gingival bleeding of over 30 % bleeding units; without taking antibiotics in the last 3 months; lack of removable orthodontic appliance.

A clinical periodontal examination was conducted

on each child in four levels depending on the adopted methodology in the Department of Pediatric Dentistry – Sofia [11]. The probing was conducted via the electronic periodontal probe 3rd generation **PA ON (Orange)**, through gingival indexes implemented in the probe's software:

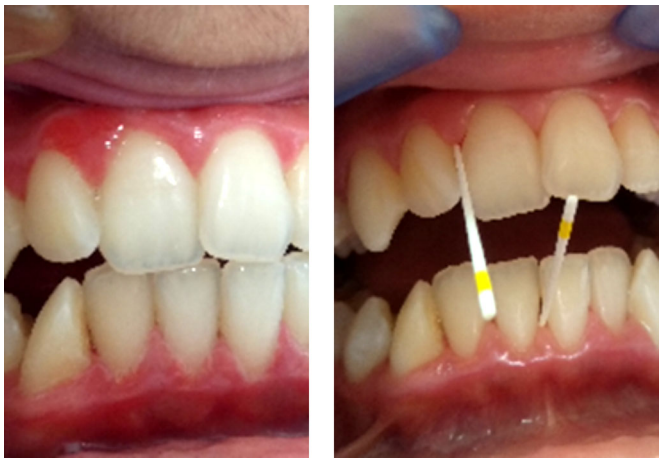
- probing depth (PD) – of all permanent teeth;
- full mouth plaque score (FMPS) – Assessment of presence/lack of dental biofilm after plaque is disclosed. All of the permanent teeth are examined in all four fields - distovestibular, vestibular, mediovestibular, oral. The index is calculated as a relative share of plaque fields;
- bleeding on probing (BOP) – Assessment of presence/lack of induced gingival bleeding via examination of all permanent teeth in four gingival units - distovestibular, vestibular, mediovestibular, oral. The index is calculated as a relative share of gingival units with bleeding;
- sulcus bleeding index (SBI) – Assessment of the severity of the gingival inflammation via examination of all permanent teeth in the first and third quadrant vestibularly, in the second and fourth – orally. One gingival unit is reported for each examined tooth on a 5-degree scale.

Genetic PCR Real Time Method

A PCR – Real Time method was used for the identification of main subgingival microorganisms and the determination of their quantities. Nine control strains were examined - *A. actinomycetemcomitans, P. gingivalis, T. denticola, T. forsythia, P. intermedia, P. (micromonas) micros, F. nuleatum, E. nodatum, C. gingivalis* (pooled sample).

Five teeth were selected (at least one per quadrant) with the greatest probing depth and the most severely inflamed – BOP index over 50 % per tooth (with bleeding in three or more points of a specific tooth) and SBI index over 2 (presence of bleeding and edema surrounding a specific tooth). A microbiological sample was taken from each tooth. The sample was taken using a sterile paper pin, which is introduced in the gingival sulcus of each separate tooth. Thereafter, the pins are collected in a joint standardized container (pooled sample), which is sent for testing (fig. 1).

Fig. 1. Taking a Microbiological Sample



RESULTS

1. Periodontal Diagnostics and Risk Assessment of the Examined Children

Level 1 – Assessment of Child's Organism

The children included in the study do not report systemic risk factors, genetic predisposition and common diseases. From the behavioral factors, it was concluded that 2/3 of the children do clean their teeth once per day, and around 90 % of the examined children clean their teeth without taking note of the time for the cleaning process.

Level 2 – Assessment of the Oral Environment

In each of the examined children, the value of the index of plaque accumulation (FMPS) is above 62 % per child, which means that more than half of the teeth surfaces examined retain plaque.

Level 3 – Assessment of Local Risk Factors

When registering the tooth status of children, no risks for periodontal health, lesions and obturations have been identified (cervical and approximal).

The children in the current study were not subjected to orthodontic treatment at the time that the study was conducted. Over half of the examined persons (55,9 %) are registered with orthodontic anomalies.

Level 4 – Assessment of the Gingiva and Gingival Sulcus

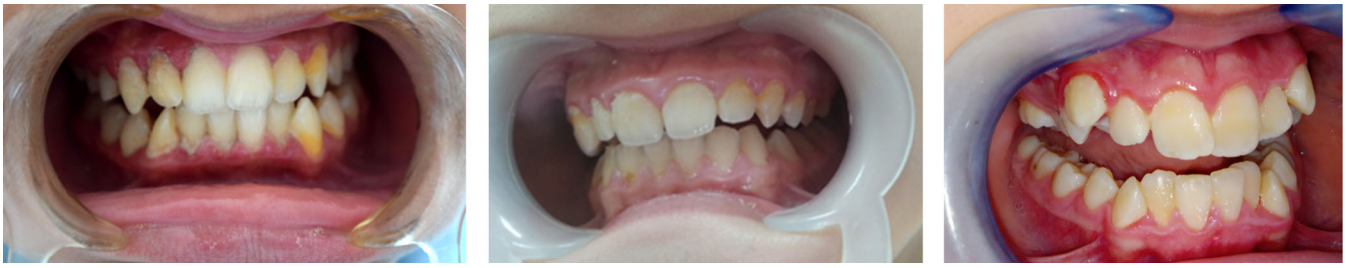
The next table represents the output values of the depth of the gingival sulcus, sulcus bleeding and induced gingival bleeding (Table 1).

Table 1. Gingival Status of the Examined Children

Gingival Status	Mean ± SD
Probing Depth	1,26 ± 0,24
Sulcus bleeding index	1.17±0.73
Bleeding on probing	52.76±17.57

From the table, it could be observed that the average depth of probing is 1,26 mm. The values obtained vary in the normal limits in children with permanent teeth. The average value of *SBI* is 1.17±0.73. These results represent a light form of gingival inflammation. The induced gingival bleeding reveals an average value of 52.76±17.57, which means that over half of the examined gingival units have bleeding. This leads to the conclusion that the spread of the gingival inflammation in all children partaking in the study is generalized, covering over 30 % of their gingival structures.

Fig. 2. Gingival Status of the Examined Children



2. Microbiological Characteristics of the Subgingival Microorganisms in Children with Plaque-Induced Gingivitis

2.1. Species Characteristics of Examined Microorganisms

In all children included in the research, identification of basic subgingival microorganisms took place (pooled sample – 9 control strains). The results are demonstrated in the next table (Table 2).

Table 2. Relative Share of the Children with Different in Species Examined Periodontal Pathogens

Microorganisms	N	Isolates (%±sp)
A.actinomycetemcomitans	4	11,8±5,53
P.gingivalis	11	32,4±8,02
T.denticola	18	52,9±8,56
T.forsythia	18	52,9±8,56
P.intermedia	24	70,6±7,81
P.(micromonas)micros	24	70,6±7,81
F.nucleatum	9	26,5±7,57
E.nodatum	11	32,4±8,02
C.gingivalis	34	100±0

The presented table reveals that in all examined children, *C. gingivalis* (green complex) – **100 %**, followed by *P. intermedia* and *P. Micros* (orange complex) – in **70,6 %**, *T. denticola* and *T. forsythia* (red complex) are isolated in 52,9 % of the children with plaque-induced gingivitis.

To that effect, *P. gingivalis* and *E. nodatum* are isolated in **32.4 %** of the children, whereas *F. nucleatum*– in **26.5 %**. The rarest in children with plaque-induced gingivitis is isolated *A. actinomycetemcomitans* – **11,8 %**.

2.2. Quantitative Characteristics of the Species Microorganisms Isolated

The average quantities of examined microorganisms vary between 1.10^3 – 1.10^5 microorganisms, with the species of the microorganisms, their quantities differ and are presented in Table 3.

Table 3. Average Quantities of Isolated Subgingival Microorganisms

Microorganisms	N	Mean ± SD	Total	
			N	%
A.actinomycetemcomitans	4	$1,5.10^3 \pm 1,5.10^3$	34	100
P.gingivalis	11	$2,0.10^5 \pm 1,3.10^5$	34	100
T.denticola	18	$8,5.10^4 \pm 1,5.10^5$	34	100
T.forsythia	18	$5,1.10^3 \pm 4,7.10^3$	34	100
P.intermedia	24	$2,3.10^5 \pm 3,6.10^5$	34	100
P.(micromonas)micro	24	$1,5.10^3 \pm 2,0.10^3$	34	100
F.nucleatum	9	$4,2.10^3 \pm 4,2.10^3$	34	100
E.nodatum	11	$5,4.10^2 \pm 4,9.10^2$	34	100
C.gingivalis	34	$1,5.10^4 \pm 3,5.10^4$	34	100

The table above reveals that the highest average value shows *P. intermedia* (orange complex) - $2,3.10^5 \pm 3,6.10^5$ and *P. gingivalis* (red complex) – $2,0.10^5 \pm 1,3.10^5$. The next in quantities are *T. denticola* (red complex) – $8,5.10^4 \pm 1,5.10^5$ and *C. Gingivalis* (green complex) – $1,5.10^4 \pm 3,5.10^4$.

The lowest average values demonstrate the representatives of the orange complex *P. Micros*– $1,5.10^3$, followed by *E. nodatum* - $5,4.10^2$.

DISCUSSION

The characteristics of the subgingival microorganisms, identified with PCR-Real Time in the current research, show that *C. gingivalis* from the green complex, according to Socransky, could be observed in 100 % of the samples taken. *C. gingivalis* represents the green complex according to Socransky and is part of the so-called early colonizers. Its role in the periodontal pathology is the creation of conditions favoring the settlement of other periodontal pathogens with pronounced virulent qualities. The role of this microorganism as an opportunistic pathogen is probably connected to the balance in the periodontal environment, and with the creation of certain conditions, it could benefit the coaggregation of more pathogenic species [12, 13].

Similar results are obtained by other researchers who isolate *C. gingivalis* in all examined children regardless of the gingival status [14]. Research carried out by Wendland et al. in children aged 15-19 with diagnosed gingivitis reveals the same regularities as those perceived by us with *C. gingivalis* [15].

The current research determined that second in terms of frequency of isolation are representatives of the orange complex according to Socransky - *P. intermedia* and *P. micros,* which are isolated in 70 % of the samples examined. The representatives of the orange complex usually settled the periodontal environment after early colonizers and are perceived as a coaggregation bridges between supra and subgingival biofilm. Contemporary research on the subgingival microflora in children with determined gingivitis shows that the prevalent microorganisms are representatives of the red and orange complex, as per Socransky. *P. gingivalis, T. forsythia* and *F. Nucleatum* are more frequently observed in children with average to severe gingivitis than in those showing slight symptoms of inflammation. *P. intermedia* could only be found in the severe forms of gingivitis [16].

Authors consider that certain not so virulent periodontal pathogenic conditions, such as *P. micros* and *P. intermedia,* could transform into key factors for the initiation of destructive inflammatory changes in the periodontium [17]. Scientific research covering 52 children with a light form of plaque-induced gingivitis isolated *P.micros, F. nuleatum* and *T. denticola* in approximately the same frequency as our research [15]. This finding in relation to the composition of the subgingival biofilm in children with plaque-induced gingivitis proves the delicate balance between initial tissue inflammation and the possibility of a relatively rapid transition to destructive inflammatory changes in the periodontal tissues.

Our research determined that representatives from the red complex, according to Socransky, are isolated in third place. In more than half of the samples taken, we isolated *T. denticola* and *T. forsythia* (52,9%). Examination of patients with gingivitis aged 19-25 ascertains that the presence of *T. denticola* in the subgingival microflora is not as much affected by the gingival status of patients. This is also confirmed by other authors who isolate it even in healthy children [5].

A distinctive combination of representatives of the orange and red complex is the possible reason for the development of an inflammatory reaction in the gingiva through potentiation of the virulent qualities of each of the mentioned periodontal pathogens. *P. gingivalis*, considered as the key periodontal pathogen, possesses a wide spectrum of virulent qualities, which lead to a disruption in the function of the immune system [19]. The correlation between the presence of *P. gingivalis* and the symptoms of inflammation in the periodontal tissues are well documented through the high levels of inflammatory mediators. The latter, on their part, lead to the creation of the ideal living conditions for the periodontal pathogens in the gingival sulcus [19, 20].

This contribution determined that the microbial number of isolated subgingival microorganisms in children with moderate plaque-induced gingivitis varies in the margin of $4{,}8.10^8 \pm 1{,}1.10^9$, at the background of which the average quantities of the examined periodontal pathogens vary between 10^3 - 1.10^5. It could be noted that the highest average value demonstrate *P. intermedia* (orange complex) - $2{,}3.10^5 \pm 3{,}6.10^5$ and *P. gingivalis* (red complex) - $2{,}0.10^5 \pm 1{,}3.10^5$, followed by the rest of the microorganisms.

Supporting our results are also the established by various authors high quantities of *P. gingivalis* in patients with more severe forms of gingival inflammation [16,18]. Popova et al. also determine that the quantities of *P. gingivalis* are high despite the fact that the concrete periodontal pathogen is less often isolated in children with gingivitis than all other microorganisms examined [14]. A number of authors are of the opinion that regardless of the mentioned qualities, the presence of *P. gingivalis* is not enough for the development of periodontitis since the multifactorial characters of these diseases is linked to risk factors different from microbial ones, which could influence the host's immune response vis-à-vis the microflora [20,21].

CONCLUSIONS:

1. In children aged 10-14 with moderately generalized plaque-induced gingivitis, there are predominantly representatives of the orange complex according to Socransky (*P. Micros* and *P. intermedia*), followed by the red complex (*T. denticola* and *T. forsythia*);

2. In children aged 10-14 with moderately generalized plaque-induced gingivitis, *P. intermedia* (orange complex) is observed in the highest quantities, followed by representatives of the red complex as per Socransky - *P. gingivalis* and *T. denticola*.

In conclusion, it could be noted that in children aged 10-14, immediately after the eruption of the permanent teeth, a subgingival microflora is formed featuring periodontal pathogens from the orange and red complexes, as per Socransky. These microorganisms participate, in large quantities, in the initiation of the inceptive forms of plaque-induced gingivitis, affecting more than half of children of this age.

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