

Examination of oral biofilm microbiota in patients using fixed orthodontic appliances in order to prevent risk factors for health complications

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Abstract

Introduction and objective. In recent decades the use of orthodontic appliances in Poland has increased; however, data on their influence on changes of components of the microbiome connected with oral biofilm are scarce. The objective of this study was to evaluate oral microbiota in terms of their role as risk factors for health complications.

Materials and method. The study included 100 patients treated with removable or fixed appliances. Oral hygiene and gingival health were determined, and periodontal swabs taken from each patient for parasitological, bacteriological and mycological microscopic and *in vitro* examinations.

Results. Oral protists and various pathogenic and opportunistic bacterial and fungal strains were identified in the superficial layer of biofilm. A higher prevalence of bacteria, *Enterococcus faecalis*, *E. faecium*, *Staphylococcus aureus* and *Escherichia coli*, and various strains of yeast-like fungi from the *Candida albicans* group, occurred in patients treated with the fixed appliance than in those using a removable appliance or not treated orthodontically. In some periodontal samples from patients treated with fixed appliances, cysts of the *Acanthamoeba* spp. were found.

Conclusions. The use of orthodontic appliances alters the status of the oral cavity; it has impact on the colonization of oral biofilm by opportunistic/pathogenic strains, and increases the risk of their dissemination to various human tissues and organs. Pretreatment examination of oral microbiome, its monitoring particularly during treatment with fixed appliances, and preventive elimination of the potentially pathogenic strains to avoid health complications, are highly recommended, especially in patients with impaired immunity.

Key words

orthodontical appliances, infectious oral microbiota, risk of health complications, prevention

INTRODUCTION

In this retrospective interdisciplinary research, the diversity in species of oral biofilm microbiota detected in patients using orthodontic appliances was analyzed in terms of their potentially infectious risk factors for health complications. It is known that the microbiome of the oral cavity may include commensals, symbiotic and potentially pathogenic species from bacteria, protists and fungi [1, 2, 3, 4, 5, 6, 7].

It should be taken into account that in recent decades the use of orthodontic appliances has increased in Poland. At the same time, no requirements have been specified to assess the risk of impact of potentially pathogenic oral microbiota on the status of the oral cavity before and during treatment with these appliances.

The basic indications for the use of orthodontic appliances are: growth modifications, malocclusion treatment, dental arch expansion, correction of minor dental discrepancies. In a healthy oral cavity, individual microorganisms and their micro-colonies adhering to soft and hard surfaces form a biofilm; in these microorganisms, synergistic and antagonistic interactions between resident species occur, including potentially pathogenic strains which remain in relative, dynamic homeostasis [8, 9, 10, 11, 12]. Fixed appliances are made of brackets, bands and orthodontic arches. Orthodontic brackets can be made of metal alloys and non-metallic materials (composites, ceramic materials), which are fixed in a specific position to the surface of the teeth by a composite adhesive. Orthodontic bands are usually cemented on the first molars. The main part of the orthodontic appliance is located in the vestibule of the mouth, or, in the case of certain types of appliances, in the oral cavity proper. The appliance components impede the self-cleaning of teeth with saliva, promote retention of food debris and

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deteriorate oral hygiene. The accumulation of plaque on components of the appliance increases. Therefore, the use of orthodontic appliances may increase the risk of developing dental caries and periodontal diseases [9, 12, 13, 14, 15].

The development of pathological changes results from the loss of homeostasis of the oral microbiome, and occurs under the influence of many endogenous and external factors, including both the microorganisms and the immunological response of the human body.

Literature data indicate that the use of orthodontic appliances influence the conditions in the oral cavity. In the blood of patients whose fixed braces were removed, opportunistic bacteria, factors of often severe local and systemic diseases were detected [16, 17, 18, 19].

Among the oral microbiota of the analyzed patients with malformations of the masticatory system, Gram-positive *S. aureus* strains [20] were frequently isolated.

The dynamics of bacterial components of the oral microbiome has been investigated for many years in various population groups. However, most of the publications concern pathogens associated with the development of caries and periodontal diseases, considered as civilization diseases; the oral microbiota, not connected with these two groups of diseases, has been studied less frequently [17, 18, 20].

Yeast-like fungi are also found in the oral cavity and are widespread in the human environment; the most common strains are *Candida albicans*, *C. glabrata*, *C. tropicalis* [12, 21, 22, 23, 24].

Two species of cosmopolitan parasitic protists: *Trichomonas tenax* and *Entamoeba gingivalis*, may occur in the oral microbiome [6, 12, 25, 26, 27, 28, 29, 30, 31], spread by direct contact during a kiss, but also indirectly by commonly used vessels.

Information on the presence of pathogenic and opportunistic microorganisms in the oral cavity is important for evaluating the health risks for patients.

OBJECTIVE

The aim of the study was to perform a comparative analysis of opportunistic/ pathogenic microbiota able to colonize the oral cavity of patients with orthodontic needs, and to assess the oral microbiota in terms of their role as risk factors for health complications.

MATERIALS AND METHOD

Comparative analysis of results of this retrospective study concerned 100 persons, aged from 6 to 23 years, who were admitted to the Department of Orthodontics of the Medical University in Warsaw during 2007–2015, and were classified into four groups:

Group I – included 25 orthodontic patients with masticatory system disorders, aged 6–13 years old, treated with removable appliances.

Group II – control group which included 25 patients, also aged 6–13 years, treated conservatively, without the use of appliances.

Group III – included 25 orthodontic patients with masticatory system disorders, aged 14–23 years, treated with fixed appliances;

Group IV – the control group included 25 patients of the same age as in Group III, 14–23-year, treated conservatively, without the use of orthodontic appliances.

All patients underwent clinical evaluation of the health status by examination of their periodontium, gingiva, presence of inflammatory processes and treatment for tooth decay. Also, for all patients, values of the Plaque Index and Bleeding Index were calculated to assess oral cavity hygiene and gingival inflammation. At the same time, the DMF Index, the common method for assessing the prevalence of dental caries and needs for dental treatment was determined, expressed as the total number of *decayed* teeth (D), *missing* (M), or *filled* (F).

Swabs were taken from 10 sites of the periodontium, dental plaques, and from dental pockets for parasitological, bacteriological and mycological examinations to isolate and identify oral microbiota [31–34]. The wet mount and permanent smears were stained with Giemsa and Trichrome to identify protists based on their morphology. To isolate fungi, Sabouraud substrate was applied, while the identification of species of *Candida* was performed with Chromagar Candida BBL tests. Preliminary microscopic identification of Gram-positive and Gram-negative bacteria strains and *in vitro* culture techniques were also applied for bacteria specific identification. The swab material was grown aerobically on bacteriological agar and on agar with 5% defibrinated sheep blood. For recovery and isolation of *Staphylococcus* strains, Chapman's plate growth medium was applied, and for identification of Enterobacteriaceae, McConkey's medium was used. The prevalence of opportunistic/ pathogenic strains detected in the oral cavities of each group of patients were determined and statistically assessed (Statistica, F-Fisher, HSD-Tukey test; $p \leq 0.05$).

RESULTS

In the clinical assessment of the oral cavities of patients treated with orthodontic appliances in Group I and III, occlusion anomalies: prognathism, retrognathism, cross-bites, dental discrepancies (crowding, rotations), as well as the accumulation of dental plaque and gingival bleeding, occurred. In all patients, tooth decay of varying intensity was detected. The clinical picture of the oral cavities of patients treated conservatively, without appliances, of control Group II and IV was generally better; however, missing teeth (in younger patients), gingival bleeding, dental caries and gingivitis were noted in some cases.

Pronounced differences in oral cavity conditions were expressed by various values of dental Plaque Index (PI), Bleeding Index (BI) and DMF among patients treated with orthodontic appliances, and those treated conservatively. The values of DMF were considerably higher in patients from Group I treated with removable appliances, compared those in Group II treated conservatively (5.6 and 3.5, respectively; $p < 0.05$). Bleeding Index was significant higher in older patients from Group III treated with fixed appliances than in those of Group IV treated conservatively (12.7 and 5.93, respectively; $p < 0.05$). Comparison of the clinical indices for all patient groups analyzed is presented in Table 1.

Examinations of microscopic and *in vitro* cultured samples of the material obtained from oral cavity swabs showed the

Table 1. Comparison of the clinical indices for all patient groups analyzed

| Age (years) | Group of patients | | DMF | Plaque Index (%) | Bleeding Index (%) |
|-------------|------------------------|-------|--------|------------------|--------------------|
| 6-13 | I removable appliances | Mean | 5.6* | 47.2 | 12.7* |
| | | range | 0-16 | 11.2-94 | 0-39 |
| | | SD | ±3.7 | ±25.45 | ±14.20 |
| | II | Mean | 3.5* | 52.2 | 12.7* |
| | | range | 0-10 | 14.6-97 | 0-26 |
| | | SD | ±2.5 | ±20.56 | ±8.07 |
| 14-23 | III fixed appliances | Mean | 9.8* | 53.4 | 12.95* |
| | | range | 1-15 | 6.3-92.6 | 0-47.2 |
| | | SD | ±3.6 | ±28.6 | ±12.43 |
| | IV | Mean | 10.36* | 49.05 | 5.93* |
| | | range | 5-18.3 | 10.8-77.4 | 0-49.8 |
| | | SD | ±3.71 | ±19.05 | ±10.40 |

* statistically significant values of clinical indices in older patients in comparison to the younger, p<0.05

presence of various pathogenic and opportunistic fungal and bacterial strains in the superficial layer of biofilm, as well as in dental pockets. In patients with malformations of the masticatory system, microbial adhesion occurred on the elements of orthodontic appliances.

Among the mouth microbiota of the analyzed patients, typical oral Gram-positive bacteriae of the *Streptococcus viridans* group were identified.

The yeast-like fungi from the *Candida* genus, predominantly various strains of *C. albicans* group, were isolated and identified in all patients groups. The greatest prevalence of these fungi was in patients of Group III treated with fixed appliances. There were statistically significant differences in data regarding patients treated and not treated with appliances.

Among bacteriae, Gram-positive of *Staphylococcus aureus* were identified. Prevalence of *C. albicans*, compared to *S. aureus* in relation to Bleeding Index in patients treated and not treated with appliances, is presented in Table 2.

Gram-positive Enterococci: *Enterococcus faecalis*,

Table 2. Prevalence of *C. albicans* and *S. aureus* in relation to Bleeding Index in patients treated and not treated with appliances

| Age (years) | Group of Patients / appliance | Patients with the microbiota: | | Bleeding Index % |
|-------------|-------------------------------|-------------------------------|----------------------------|------------------|
| | | <i>C. albicans</i> % /number | <i>S. aureus</i> % /number | |
| 6-13 | I / removable appliance | 12 / 3 | 8 / 2 | 12.7* |
| | II / without appliance | 8 / 2 | 4 / 1 | 7.4* |
| 14-23 | III / fixed appliance | 24* / 6 | 16* / 4 | 12.95* |
| | IV / without appliance | 8* / 2 | 8* / 2 | 5.93* |

*Level of statistical significance set at p < 0.05.

There are statistically significant differences in data regarding patients treated and not treated with appliances.

E. faecium, and also Gram-negative Enterobacteriaceae: *Escherichia coli*, *Enterobacter cloacae*, *Pantoea agglomerans*, *Klebsiella sp.*, were detected in various patient groups. Comparison of the prevalence of selected Gram-positive and Gram-negative bacteriae from the oral cavities of 14-23-year-old patients is presented in Table 3.

In the material obtained from oral cavity swabs, live protozoans: *Trichomonas tenax* and *Entamoeba gingivalis*

Table 3. Comparison of prevalence of selected Gram-positive and Gram-negative bacteriae from oral cavities of 14-23-years old patients in relation to Bleeding Index

| | patients with bacteriae | |
|--|-------------------------|---------------------|
| | Group III number / % | Group IV number / % |
| Enterococci <i>E. faecalis, E. faecium</i> | 8 / 32* | 3 / 12* |
| Enterobacteriaceae <i>P. agglomerans, E. coli</i> | 5 / 20* | 2 / 8* |
| Bleeding Index in patients of | | |
| | Group III | Group IV |
| Mean | 12.95 * | 5.93 * |
| range | 0-47.2 | 0-49.8 |
| SD | ±12.43 | ±10.40 |

* Statistically significant differences in data regarding patients treated and not treated with appliances, p<0.05

were also identified in the older patients, with the low prevalence within the range of 4-8%.

In some periodontal samples from three patients (12%) of Group III, double-walled cysts were found and identified as cysts of the amphizoic amoebae – facultative parasitic *Acanthamoeba* spp..

DISCUSSION

Comparative qualitative analysis of results of this research revealed clear differences in the prevalence of microbiota strains detected in the oral cavities of particular patient groups.

It is considered that the oral cavity consists of polymicrobial communities, heterogeneous in origin, that can include various endogenous and exogenous species; complex interrelations occur between multilayer components of biofilm, particular species of oral microbiota, and the host organism. Moreover, according to the result of studies involving the oral microbiota metagenomic projects, the oral cavity is one of the most taxonomically diverse sites of the body, in which some infectious species have been identified as protists, fungi and bacteriae [2, 3, 4, 33, 34].

During the last 20 years in European countries, including Poland, in particular the prevalence of fungal infections has significantly increased. Yeast-like fungi of the *Candida albicans* group are widespread in the human environment; they enter the body through damaged mucous membrane, skin, by inhalation, food and sex. Simultaneously, the components of the fungal cell wall and products released by their cells can inhibit an immune response, which is a serious health risk, even in people with an efficient immune system [8, 21, 23, 35, 36, 37]. A high density of fungi in the mouth, throat and larynx is a serious risk for the spread of fungal diseases.

In the current study there was a clear correlation between the use of fixed appliances and the frequency of isolation of *Candida* sp. The intensity of *C. albicans* was correlated with higher values of platelet and bleeding indices, indicating poor oral hygiene and occurrence of periodontitis.

At the same time, in orthodontic patients using fixed appliances, various potentially pathogenic bacteriae of Streptococci and, also Enterococci groups were detected.

It should therefore be taken into consideration that the oral cavity may act as a reservoir/source of etiological agents of local infections (abscesses), and also disseminated infections of the urinary tract, bile ducts, endocardium, among others, including nosocomial diseases [7, 20, 30, 32].

The parasitic protozoa *Trichomonas tenax* and *Entamoeba gingivalis* were rarely included in clinical studies of the masticatory system; some authors describe them as harmless commensals, despite evidence of the pathological effects of these protists. The destructive impact of these protozoa on oral tissues was detected as their adhesive capacity, high extracellular activity of their proteinases, and distribution of various types of collagen, as well as haemolytic activity on the mucous membrane and other structures of the masticatory system. The protists do not belong to resident microorganisms; they colonize the oral cavity due to invasion that may result in periodontitis, paranasal sinuses. Trichomonads were detected in the content of pulmonary abscesses [25, 26, 27, 28, 29, 31, 38, 39].

It is noteworthy that the *Acanthamoeba* spp. cysts were also detected in the oral cavities of several patients treated with fixed orthodontic appliances. The results are in agreement with several previous findings [12, 30, 39]. Various strains of *Acanthamoeba* sp. are worldwide facultative parasites that may cause serious threats to human health as etiological agents of granulomatous amoebic encephalitis and vision-threatening *Acanthamoeba* keratitis.

Amoebae are isolated from the hospital environment, among others, as contaminants of surgical instruments, the dental irrigation system, equipment and accessories in health facilities [30, 39, 40].

CONCLUSION

In the available literature, data is scarce on the prevalence of various opportunistic bacteria, parasitic protozoans and yeast-like fungi identified in the oral cavity of people with masticatory system disorders treated orthodontically.

The presented comparative qualitative analysis has shown that the use of orthodontic appliances alters the status of the oral cavity, and impacts on its colonization with different opportunistic/ pathogenic strains.

It should be emphasized that the human oral cavity may act as a major, yet poorly known, reservoir of microorganisms that can induce clinically important infections.

Moreover, as knowledge about the risks generated by components of the oral microbiome is still insufficient, further studies are important to decrease the risk of health complications.

Pretreatment examination of oral microbiota, its monitoring, especially during treatment with fixed appliances, and preventive elimination from mouth of the potentially pathogenic strains to avoid health complications, are highly recommended, especially in patients with impaired immunity.

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