

## Original papers

# Evaluation of selected oral cavity microbiota – risk factors of management complications in patients with masticatory system disorders

Paweł J. Zawadzki<sup>1</sup>, Konrad Perkowski<sup>2</sup>, Bohdan Starościak<sup>3</sup>, Monika Dybicz<sup>4</sup>, Wanda Baltaza<sup>5,6</sup>, Krzysztof Pionkowski<sup>5</sup>, Lidia Chomicz<sup>5</sup>

<sup>1</sup>Clinic of Cranio-Maxillo-Facial and Oral Surgery and Implantology, Medical University of Warsaw, Lindleya 4, 02-005 Warsaw, Poland

<sup>2</sup>Department of Orthodontics, Medical University of Warsaw, Nowogrodzka 59, 02-005 Warsaw, Poland

<sup>3</sup>Department of Pharmaceutical Microbiology, Medical University of Warsaw, Oczki 3, 02-007 Warsaw, Poland

<sup>4</sup>Chair and Department of General Biology and Parasitology, Medical University of Warsaw, Chałubińskiego 5, 02-004 Warsaw, Poland

<sup>5</sup>Department of Medical Biology, Medical University of Warsaw, Nowogrodzka 73, 02-018 Warsaw, Poland

<sup>6</sup>Department of Disaster Medicine, Warsaw Medical University, Zwirki and Wigury 81, 02-091 Warsaw, Poland

Corresponding Author: Paweł J. Zawadzki; e-mail: pawel.j.zawadzki@gmail.com

**ABSTRACT.** The retrospective analysis of data on oral cavity clinical status in relation to microbiota species composition is presented. The research regards patients of different age, with and without congenital malformation, pretreatment assessed for occurrence of pathological changes in the masticatory system. Samples of the swabs collected from each patient (from dental plaque, periodontium and dental pockets) were used for identification of oral protozoans in wet slides and stained preparations; additionally, transmission electron microscope examination was performed. The material was used for *in vitro* cultures to identify bacteria strains. Clinically, intensity of tissue deteriorations was higher in patients with a congenital disease. Alive *Trichomonas tenax* and *Entamoeba gingivalis*, species with confirmed pathogenic impact on oral cavity and neighboring structures, were detected with higher prevalence in older patients. *Enterococci*, *Staphylococcus aureus*, various Enterobacteriaceae were more frequently detected in patients with somatic and mental retardations; in mouths of those patients, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* opportunistic strains occurred. Masticatory system abnormalities favor colonization of oral cavity by exogenous species and dissemination of infections, especially dangerous for patients with congenital diseases. Oral microbiota assessment and preventive measures may be helpful to avoid subsequent peri-surgery complications.

**Key words:** oral parasites, bacteria, masticatory system disorders

## Introduction

The oral cavity creates an open environment, which includes many endo- and exogenous components. Among microbiota that can colonize this environment, resident species of *Streptococcus viridans* group, typical for healthy mouth, usually occur. The species composition of oral microorganisms depends on various biotic and abiotic factors. Poor oral hygiene, drug-induced overgrowth of gingiva, changes in pH and chronic disease

predispose to pathological changes emergence [1–3]. To date, the species composition of pathogenic oral microbiota that may influence medical treatment complications in patients with chronic disabilities associated with the congenital and multi-factorial diseases was not extensively investigated. The disturbance of complex, dynamic oral cavity homeostasis that results in changes of mouth ecology and composition of microorganisms, may promote infections with the exogenous species, bacterial and protozoan pathogens.

Our previous studies of kidney allograft recipients under chronic immunosuppression, patients with diabetes mellitus insulin treated and some with mental retardation showed that decreased resistance and altered pH linked with the systemic diseases are, among others important factors associated with deterioration of periodontal tissues and infections with parasitic protozoans and other microorganisms [3–7].

This work is subsequent to comparative holistic investigations involving different populations aimed to determine the risk of peri-operative infections. They involved different population groups, including patients with systemic diseases, particularly in aspect of a relation between the oral cavity status and ethiopathogenic role of microorganisms that can occur in oral environment.

In this study, we analyze retrospectively the species composition of oral parasites and bacteria in term of evaluation of the selected microbiota as risk factors of management complications in patients with masticatory system deteriorations mentally disordered in comparison to those without congenital diseases routine dental treated.

## Materials and Methods

The present retrospective study includes 96 persons, men and women with and without congenital diseases, treated 2005–2012 in Clinics and Departments of Medical University of Warsaw, pertained to oral cavity microbiota. The patients have been classified into two groups: Group I – involving forty eight persons with congenital diseases, associated with mental retardation and masticatory system disorders, before specific treatment and Group II – involving forty eight persons without congenital diseases with routine dental treatment.

The patients have been additionally categorized into two age groups: 28 to 40 and 41 to 53 years. All analyzed patients were clinically assessed for general health, their pretreatment oral cavity condition: the status of periodontal tissues, dentition, oral hygiene and, particularly, for the occurrence of pathological changes in the masticatory system.

Swabs from ten sites on surface of dental plaques, periodontium and dental pockets collected from each patient were immediately placed in sterile tubes containing the physiological salt solution, pH 6.8 and incubated at 36°C.

Samples of the material were used for preliminary identification of oral protozoans in wet slides; next, Giemsa and trichrom-stained preparations were made for the light microscope studies in term of verification of parasitic protozoan species.

Some of the incubated samples deriving from different oral cavity sites after centrifugation procedure were used for electron microscope examinations. The isolates were fixed in a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4 for 2h at room temperature. The material was post fixed in 1% osmium tetroxide for 2h, dehydrated in a graded ethanol series, infiltrated with propylene oxide and embedded in Spurr's epoxy resin. Semithin sections were preliminary examined in the light microscopy; ultrathin sections double stained with lead citrate and uranyl acetate were examined with TEM (a transmission electron microscope, JEM 1200EX).

Simultaneously, the material was also used for *in vitro* cultures to isolate bacteria; isolates grown on bacteriological agar and on agar with 5% defibrinated sheep blood were routinely tested for specific identification of bacteria.

The species composition of oral parasites and bacteria as well as prevalence of microbiota found in oral cavities of all patient groups were assessed; statistical analysis was carried out by the program Statistica; F-Fisher and HSD Turkey tests were applied for an assessment of statistical significance ( $p \leq 0.05$ ).

## Results

**Clinical assessment of the oral cavity condition.** The retrospective study showed differences in oral cavity conditions of analyzed patients expressed by various extension of masticatory system disorders. In Group I involving persons with mental retardations of different in origin, having difficulties with the maintenance of good oral hygiene, requiring clinical treatment for dental conditions, soft tissues and stomatognathic abnormalities linked with the main congenital disease were found, such as maxillary prognathia, retrognathia or micrognathia, dental transposition, open mouth, drug-induced mucosal inflammation and gingivitis. The poor oral hygiene status expressed by advanced dental caries, caries lesions as well as gingival bleeding, periodontitis and teeth looses was noted. The masticatory system disorders



Fig. 1. The patient of Group I with masticatory system disorders – the dental transposition and periodontitis



Fig. 2. The patient of Group I with masticatory system disorders – the advanced dental caries and gingivitis

– smooth tissues and stomatognathic deteriorations, with advanced dental caries in some patients of Group I are illustrated in Figs. 1 and 2.

In Group II involving persons without congenital diseases treated conservatively, the oral cavity status was a much more favorable, however dental caries, teeth looses and gingival bleeding were found in some cases. It should be underlined, that the clinical changes were much more intense in the mentally-retarded patients and those categorized into age group 41 to 53 old years.

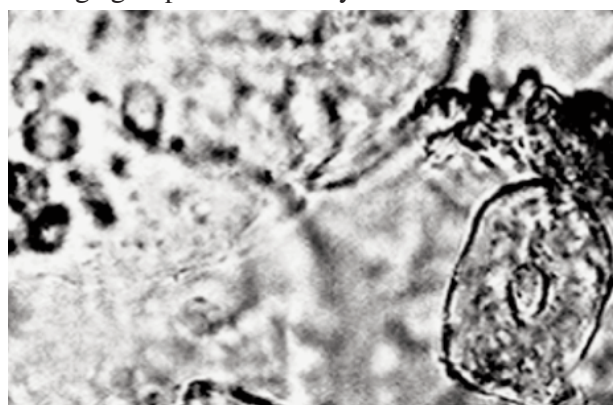


Fig. 3. Micrograph of wet preparation: *T. tenax* trophozoites between oral cavity cells

Table 1. Comparison of the prevalence of parasitic protozoan infections in particular patient's groups

Group	Age of patients	Patients infected with <i>T. tenax</i> number/%	Patients infected with <i>E. gingivalis</i> number/%
I	28–40	8/33.3	3/12.5
	41–53	14/56.6	7/29.2
II	28–40	3/12.5	2/8.3
	41–53	4/16.6	2/8.3

Table 2. Comparison of the prevalence of bacterial strains in particular patient's groups

Group	Age of patients	Patients with oral cavity colonized by bacteriae:		
		<i>E. coli</i> number/%	<i>S. aureus</i> number/%	<i>P. aeruginosa</i> number/%
I	28–40	8/33.3	3/12.5	2/8.3
	41–53	14/56.6	12/50	5/20.1
II	28–40	2/8.3	1/4.1	0
	41–53	3/12.5	1/4.1	0

**Microbiota detected in oral cavities of patients from particular groups.** The light microscopic and TEM examinations as well as *in vitro* tests of the isolates deriving from oral cavities of the analyzed patients revealed the presence in the superficial layer of periodontium and in the dental pockets various microbiota including parasitic protozoan species and different bacterial strains.

**Parasitic protozoans.** In all patient groups alive flagellates and amoebae were detected in wet slides, identified basing on their morphology in stained preparations as *Trichomonas tenax* and *Entamoeba gingivalis*. The prevalence of the protozoans varied in relation to type of patient group and the patient age.

The *Trichomonas tenax* trophozoites were isolated from different oral cavity sites and in saliva. The trichomonads were more frequently present in older persons with congenital diseases, associated with mental retardation and somatic deterioration than in the 28 to 40 years old patients; simultaneously, the persons without congenital diseases treated conservatively were less frequently infected with the flagellates. *T. tenax* trophozoites between oral cavity cells are visible in micrograph of wet preparation of oral swab (Fig. 3).

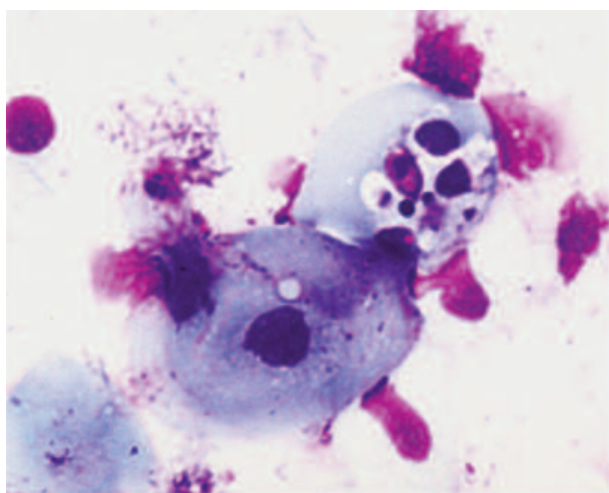


Fig. 4. Micrograph of trichrom-stained slide: *E.gingivalis* trophozoite and oral cavity cell

The *Entamoeba gingivalis* trophozoites were mainly found in dental pockets and on the surface of periodontium; no amoebae were detected from saliva. The oral amoebae were more frequently isolated in 41 to 53-year old patients with congenital diseases than in those treated conservatively. The *E. gingivalis* trophozoite and oral cavity cell are visible in micrograph of trichrom-stained slide (Fig. 4). Generally, the prevalence of *E. gingivalis* was lower than *T. tenax*. Comparison of the prevalence of protozoan infections in particular patient's groups is presented in Table 1.

**Bacterial strains detected in oral cavities.** In all patients analyzed, some bacterial species of *Streptococcus viridans* group, typical inhabitants of the human oral cavity were found, which, however were not a subject of this evaluation.

Comparative analysis of the material from swabs, prepared for microscopic studies, *in vitro* cultured and tested, revealed the presence of Gram-positive and Gram-negative bacterial strains occurring with different extensiveness in various oral cavity sites.

*Enterococcus faecalis*, *E. faecium* and *Micrococcus lutens* as well as *Staphylococcus aureus* strains belonging to Gram-positive bacteria were more frequently detected in the patients with somatic and mental retardations, which indicated more intense deteriorations in masticatory system than in those without the congenital diseases.

The highest prevalence of fecal bacteria was detected in older patients of Group I.

Among Gram-negative bacteriae, *Escherichia coli* and *Enterobacter agglomerans* were found also in material from both patient group.

Simultaneously, only in oral cavities of patients of Group I *Klebsiella pneumonia* and *Pseudomonas aeruginosa*, bacterial strains usually inhabiting the deeper regions of human intestine were detected.

Comparison of the prevalence of *E. coli*, *S. aureus*, *P. aeruginosa* strains in particular patient's groups is presented in Table 2.

## Discussion

Literature describing the changes in species composition of oral microbiota in patients with congenital disabilities and systemic disorders is scarce [8–10].

In our previous studies, a clear interrelation between oral tissues deterioration and alteration of oral cavity ecology, leading to changes in microbiota species composition, were revealed in patients with decreased resistance: the insulin treated persons with diabetes mellitus, chronic hemodialyzed patients as well as kidney allograft recipients [4,6,7].

Simultaneously, a role of the microbiota as potential factors for peri-surgery complications was assessed [11,12].

Results of other investigations indicated that occurrence of symptoms of periodontium or gingival deteriorations correlated with colonization of oral cavity by parasitic protozoans [3–5,13–18]. It was in accordance with evidences of pathogenic impact of *Trichomonas tenax* and *Entamoeba gingivalis* trophozoites on oral cavity tissues and neighboring structures at biochemical level [19–22].

It is emphasized that oral health has a great impact on patients with mental illness for whom an access to dental care is poor. The results of the first known study of local and national oral health needs of patients with mental illness indicated that they differ from the general population: the oral health in this specific population group is poorer than in general population [10].

## Conclusions

Results presented in this analysis indicated that masticatory system disorders, especially in persons with congenital disease may favor alteration of oral microbiota homeostasis.

It can change a local oral cavity ecology and result in a colonization of the mouth by exogenous opportunistic and pathogenic species, bacteria and



protozoans. The patients with congenital disease are at major risk for development of not only local infections, but also of subsequent dissemination of pathogenic microorganisms from oral cavity to other organs.

As it can impact general health status as well as effectiveness of treatment management, a proper assessment of oral cavity microbiota before therapy, adequate preventive measures are essential to avoid co-infections and subsequent peri-surgery complications.

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