

Original papers

Comparative assessment of selected intraoral microorganisms – potential factors for peri-surgical management complications

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ABSTRACT. In this research, a comparative analysis of results of investigations involving different human populations, in terms of a relation between the oral cavity health and the species composition of mouth microbiota is reported. The purpose of this analysis was to identify and assess microorganisms that could cause health complications in patients with neoplasm requiring dental problem-related surgical management. The patients with the oral cancer surgically treated and those without neoplasm were assessed for their oral health: status of teeth, gingiva, periodontium, and occurrence of inflammatory processes. From each patient, microorganisms isolated of periodontium, dental plaque, and dental pocket swabs were identified in wet and stained microscopic preparations; standard microbiology *in vitro* techniques were also applied to determine the fungal and bacterial strains. The comparative analysis of results of direct microscopic examinations and *in vitro* cultures assessment indicated significant differences in prevalences of fungi, parasitic oral protozoans and bacteriae in particular patient's groups. Yeast-like fungi belonging to *Candida* genus, mostly of *C. albicans* group, were identified in 93.75% patients with the oral cancer, while in 25% of individuals assessed without neoplasm. *E. gingivalis* amoebae were only found in 12.5% patients with the serious disease; no trichomonads were detected in all patients analyzed. Among bacteria species, potentially pathogenic Enterobacteriaceae were found in the patients with oral cancer. The pronounced shift in the microbiota species composition in the patients who needed prolonged treatment due to oral cavity cancer, compared to other generally healthy persons has been showed in this analysis.

Introduction

In last the two last decades, components of oral cavity microbiota, their collective genome including, and a coexistence of particular species in the oral cavity environments are more frequently investigated [1–7]. The interrelations between the oral microbiome organisms in the healthy individuals, and associations with various local/general metabolic deteriorations and chronic diseases are the important objective of studies, also undertaken in our investigations [8–13]. Particularly, a role of biofilm in regard to

interrelations between microbiota components, and mouth tissues is recognized. This complex structure may directly affect oral cavity tissues and simultaneously indicates a higher resistance to antimicrobial agents than free mouth microorganisms, out of biofilm. At the same time, studies have been undertaken to estimate the associations between oral microbiome and biofilms, and oral pre-cancers and cancers [14–20].

In our previous studies, involving patients with decreased immunity, from different human populations: the insulin treated patients with diabetes mellitus, chronic hemodialyzed, mentally

disordered, various microorganism strains, potential risk factors of local and general infections, were detected in their oral environments. There were differences in oral species compositions in the comparison to those revealed in generally healthy persons [8–11,13].

The purpose of this retrospective analysis was to evaluate mouth microbiota that could cause health complications in the oral cancer patients with dental – related problem requiring surgical management.

Materials and Methods

The retrospective analysis includes 16 persons, men and women, aged between 60–75 years, admitted to Clinics and Departments of the Medical University of Warsaw (2014, 2015), with oral cancer, requiring dental problem-related surgical management (Group I), and 16 individuals in the same age range, without tumors (Group II). In all patients, the clinical status of their teeth, periodontium, gingiva, and occurrence of inflammatory processes was assessed. From each patient, swabs from 10 sites of periodontium, dental plaques, and dental pockets were collected and performed according to the previously described procedure [11]. The wet and permanent smears, stained with Giemsa and trichrome, were prepared for qualitative microscopic assessment of parasitic oral protozoans that have been identified on the basis of their morphology. Standard microbiology *in vitro* techniques were applied to detect the bacteriae and fungi. The bacteriological agar and agar with 5% defibrinated sheep blood, Chapman's plate growth medium for recovery and isolation of *Staphylococci*, and McConkey's medium to detect of Enterobacteriaceae were used; Sabouraud substrate and Chromagar Candida BBL plates were applied to identify fungi. The species composition and prevalence of particular microbiota: fungi, oral parasitic strains, and bacteriae, detected in oral cavities of patients with and without oral cancer were assessed, compared and statistically analyzed.

Results

The retrospective assessment showed different pathological changes in the oral cavities of all 60–75 year patients included in this analysis. Disorders of the oral health were expressed by a various extension of soft tissues and stomatognathic system deteriorations, e.g. the poor mucous

membrane status, dental caries, caries lesions, pathological pockets, loose teeth, gingival bleedings, advanced periodontitis. The symptoms were much more intense in patients with the main disease: tongue, jaw or lower jaw cancer requiring prolonged therapy, including surgical treatment.

Examinations of direct microscopic and *in vitro* cultivated oral isolates, both revealed the typical oral Gram-positive bacteriae of *Streptococcus viridans* group, typical resident species related with caries and periodontal diseases, in all examined patients. Miscellaneous microbiota, including fungal species, parasitic protozoans and various bacterial strains, were detected in the superficial layer of periodontium and in the dental pockets, also.

Comparative qualitative analysis indicated various prevalence of fungi, protozoans, and bacteriae in oral cavities of particular patient groups. Yeast-like fungi, mostly of *C. albicans* group, were noted with high intensity and the highest, 93.75% frequency in men and women with the oral cancer, and were less frequently found, with 25% prevalence in persons without neoplasms.

Among protozoans, in wet slides of swab material, collected from different oral cavity sites, alive amoebae were found sporadically; they were identified in stained slides, by their morphology, as *Entamoeba gingivalis*. The amoebae were detected sporadically and only in the patients with the oral cancer disease, with 12.5% frequency, whereas no trichomonads were found in all patients analyzed.

In cultured isolates taken from oral cavities of analyzed individuals, Gram-positive and Gram-negative bacteriae were also found in several patients. Among Gram-positive strains, *Enterococcus faecalis* were noted. Data obtained from specific growth medium examination showed presence of Gram-negative Enterobacteriaceae identified as *Escherichia coli*, *Enterobacter agglomerans*, *Enterobacter* sp.

The comparison of prevalence of potentially pathogenic microbiota found in oral cavity of patients of particular groups is presented in Table 1.

Discussion

Results of our comparative analysis showed significant differences in prevalence of microbiota components detected in oral cavities of assessed patients, all ranging from 60 to 75 years of age. These differences were expressed by higher

Table 1. Comparison of prevalences of potentially pathogenic microbiota in oral cavity of patients of particular groups

Microbiota	Group of patients	Patients with the microbiota number / %
Fungi:		
<i>Candida albicans</i> group	I	15/93.75%
	II	4/25%
Protista:		
<i>Entamoeba gingivalis</i>	I	2/12.5%
	II	0
Bacteriae:		
<i>Escherichia coli</i>	I	2/12.5%
	II	0
<i>Enterobacter agglomerans</i>	I	1/6.25%
	II	0
<i>Enterococcus faecalis</i>	I	2/12.5%
	II	1/6.25%

prevalences of all microbiota found in patients surgically treated, under prolonged therapy, compared to generally healthy dental patients, without indications for surgical procedures.

The diversity in the composition of the bacterial and fungal microbiota, in community and in biofilms of each intraoral habitat (tooth surface, lateral and dorsal tongue), were reviewed by several researchers; it reflected, most likely, the different surface properties and microenvironments associated with oral cancers [18–20].

In this retrospective study, the diversity of composition of the oral microbial community and in prevalences of particular fungal, protozoan and bacterial strains, was also revealed.

Result of our analysis indicated that the incidences of colonization of surgically treated cancer patients with fungi of *Candida albicans* group were very high in comparison to persons without tumors.

Yeast-like fungi are widespread in the human environments, and occur outside and within human body [3,7,16,21–23]. They can colonize the oral cavity, both in the early and later stages of ontogenetic development. Despite the significant

discrepancies in available data on incidences of *Candida*, given by different authors, there is a consensus for the frequent detection of these fungi in persons with a healthy oral cavity. However, the candidosis, an inflammatory disease, usually develops as endogenous opportunistic infection; as it reports Ghannoum et al. [3], “it is possible that the presence of a given fungal isolate... could be the first step in predisposing the host to opportunistic infections”. Among factors contributing to an increased risk of the candidosis referred by many authors are: immunodeficiencies, immunosuppression, a surgery, the antibiotic and cytostatic therapies; during invasive procedures, fungi may enter the blood system and be spread to different organs. Recent literature data indicate that a threat of fungal disease, often severe, remains a major clinical, diagnostic and therapeutic problem [18–24].

Two parasitic protozoans may colonize the oral cavity of humans: *E. gingivalis* and *T. tenax*. Our analysis showed the presence of *E. gingivalis* amoebae only in surgically treated patients with cancer; moreover, no trichomonads were found in all patients involved in the our research.

Protozoans are rarely taken into consideration in clinical researches of the masticatory systems. Literature data available from many countries, including Poland, shows that the amoebae are mainly detected in patients with extensive caries, advanced periodontal disease, in periodontal swabs from the mucous membrane, from pathological pockets. It was demonstrated, that various *E. gingivalis* prevalences depend on population groups; also, the increase of amoeba frequency occurs with advanced age of patients [25–30].

Between bacteria species, apart from typical resident species related with caries and inflammatory periodontal diseases, potentially pathogenic Enterobacteriaceae were found in patients with oral cancer. Gram-negative Enterobacteriaceae strains, *Escherichia coli* and *Enterobacter agglomerans* are known as causative agents of serious infections, particularly dangerous for immunocompromised and elderly persons. Among *E. coli* rods, enterotoxic, enteropathogenic, enterohemorrhagic strains may cause nosocomial infections and sepsis.

Gram-positive bacteriae of *Enterococcus faecalis* strains were also found in oral cavities of several patients included in this retrospective study. The bacteria strains may be causative agents

stomatopathy, pneumonia, nosocomial urinary tract infections, especially dangerous for immune-compromised and elderly persons.

The findings confirmed the literature and own data obtained for other population groups in regard to an influence of various systemic diseases, and host aging factors, in different degree, on occurrence in the oral cavity of potentially pathogenic bacterial strains [4,8,10–13,17].

Conclusions

In this comparative analysis, potentially pathogenic yeast-like fungi, parasitic oral protozoans and different bacteria species were identified in oral cavities of patients requiring a dental problem-related treatment. The diseases of the oral cavity, particularly in elderly patients with cancer, had an impact on alteration of oral cavity homeostasis and changes of the mouth microbial community; thus the pronounced shift of the microorganism species composition was present in these patients, in comparison to these generally healthy persons.

Increased levels and high densities of opportunistic species – components of oral microbiota impede the treatment especially in patients with oral cancer and cause threat of increased risk of subsequent disseminated infections from oral cavity to other organs.

It should be emphasized that advances in knowledge about a diversity and coexistence of potentially pathogenic strains within the oral cavity, is clinically important to avoid the risk of development of serious peri-operative complications.

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