Assessment of the Oral Microbiome in the Development of Recurrent Oral Aphthae in Patients with Gastrointestinal Pathology

Irina Nikolaevna Usmanova[1], Irina Aleksandrovna Galimova[1], Larisa Pavlovna Gerasimova[1], Fatima Yuryevna Daurova[2], Elena Rafilovna Abdrakhmanova[1], Rauza Fazylovna Khusnarizanova[1], Irek Ramimovich Usmanov[1]

Pren. Méd. Argent.
October 2019
Vol. 105 - Nº 9
621-627

ABSTRACT

Chronic recurrent oral aphthae in residents living in an ecologically unfavourable region are characterized by a permanent course and prolonged recovery processes of regeneration of pathological elements of the oral mucosa. Using the microbiological method and modern test systems, it has been found that on the surface of aphthae an extremely diverse state of the oral microbiota is determined and its types are diverse. Trigger mechanisms have been determined. The role of representatives of various types of microorganisms - enterococci, staphylococci, streptococci, yeast-like fungi of the genus Candida (C. albicans) and obligate-anaerobes in the development of recurrent oral aphthae has been established. The data obtained can serve as an indication for the development of modern treatment and preventive measures regarding this category of patients.

Keywords: *oral microbiome, recurrent oral aphthae, oral mucosa.*

Introduction

Around the world every year there is an increase in the number of patients with somatic diseases, including gastrointestinal diseases [1-3]. The oral mucosa pathology is one of the most complex, pressing problems of modern dentistry. However, to date they have been the least studied in relation to the impact of etiological, pathogenetic factors and the state of general somatic status [4-6]. The problem is further complicated by the fact that no specific treatment and preventive measures have been developed so far to reduce their prevalence [6]. Certain manifestations of pathology of the mucous membrane of mouth, tongue, lips most often differ in a chronic relapsing course, occur with severe clinical symptoms against the background of the development or worsening of somatic diseases, which leads to a decrease in the quality of patients' life [4-8]. Therefore, patients with gastrointestinal diseases and the mucous membrane of mouth and tongue involved in the pathological process need a thorough comprehensive dental examination and the continuity of related gastroenterologists and therapists, immunologists [9-11].

The microorganisms making up the microbial biocenosis of the oral cavity are represented by an average of 530 to 700 species, among which are such prevailing bacteria as Actinomyces, Campylobacter, Capnocytophaga, Corynebacterium, Fusobacterium, Neisseria, Granulicatella, Prevotella, Streptococcus, Veillonella, Fusobacterium, Porphyromonas, Prevotella, Tannerella, Treponema, constantly existing bacteria Candida albicans, C. tropicalis, C. pseudotropicalis, C. guilliermondi, viruses, protozoa, mycoplasmas, etc. The state of the microbial biocenosis of the oral cavity and the body as a whole is directly dependent on various factors, while their negative impact exceeds compensation abilities of the ecological system "macroorganism - normal flora", at the same time there occur violations, which are most often a trigger mechanism for the development and maintenance of various pathological conditions in the oral cavity and in the body

1 Bashkir State Medical University of the Ministry of Health of Russia, Lenina St. 3, Ufa, 450008, Russia
2 Peoples' Friendship University of Russia, Miklukho-Maklaya St. 6, Moscow, 117198, Russia

as a whole [12, 13]. With the emergence of molecular-genetic research methods, the possibilities of its study have expanded. As a rule, various biotopes containing biological material and reflecting the state of a certain microbial community are examined in the oral cavity: oral fluid (saliva), gingival fluid, plaque from the surface of affected elements - aphthae and ulcers, soft and hard plaque, the contents of periodontal and periodontal pockets. Moreover, all these biotopes, with the exception of periodontal pocket biocenosis, are extremely unstable and significantly depend on the state of oral hygiene.

A number of studies have shown the relationship between the oral microbiome and the digestive system, which is manifested in the dominance of such periodontal microorganisms as Porphyromonas gingivalis, Treponema denticola, Streptococcus mutans, Streptococcus oralis, Streptococcus salivarius, Streptococcus sanguis, Streptococcus macacae and Streptococcus sobrinus. Therefore, it is important not only to know the quantitative and qualitative composition of the microbial biocenosis of the oral cavity, but also its state in case of gastrointestinal diseases, which determined the relevance of this study [5, 10].

Thus, as for patients with chronic gastrointestinal diseases and dental pathology, the use of integrated effective diagnostic schemes is important, which will contribute to the further planning of unified approaches to local and general treatment and management of patients, which determined the relevance and purpose of this study.

Material and methods

To solve the tasks, the authors performed a comprehensive dental examination of 250 individuals (average age 32,2 ± 1,4 years old), recurrent oral aphthae were detected in 80% of cases, while gastrointestinal diseases prevailed in the structure of general somatic pathology of this category of people.

To study the species composition of microflora in all patients with recurrent aphthae, the clinical material was collected from the surface of the affected elements (aphthae, ulcers). To separate facultative anaerobic microorganisms, seeding was carried out on special Hi-Crome series differential diagnostic media. Part of the initial clinical material was tested on a PCR analyzer and parallel screening was performed under aerobic and anaerobic conditions of the remaining part of the material from the given media onto HiCrome series differential diagnostic media for further cultivation and subsequent identification of microorganisms. PCR testing of clinical material was carried out in real time mode using a test system "Dentoscreen" for 6 periodontopathogens: Porphyromonas gingivalis, Treponema denticola, Aggregatibacter actinomycetemcomitans, Tannerella forsythensis, P. endodentalis, Fusobacterium nucleatum, Prevotella intermedia. The advantage of PCR diagnostics is the ability to detect both viable bacteria and DNA fragments of microorganisms in the studied clinical material.

Statistical processing of the data obtained was carried out on a personal computer IBM PC/AT with the use of Statistica 7.0 software package and Excel 2007 spreadsheets. Student's t-test was used to compare the data. The level of reliable significance was $p \leq 0.05$.

Results and discussion

To solve the tasks, the authors performed a comprehensive dental examination of 250 people with gastrointestinal diseases of the Therapeutic Department of the Clinic of the Federal State Budgetary Institution of Higher

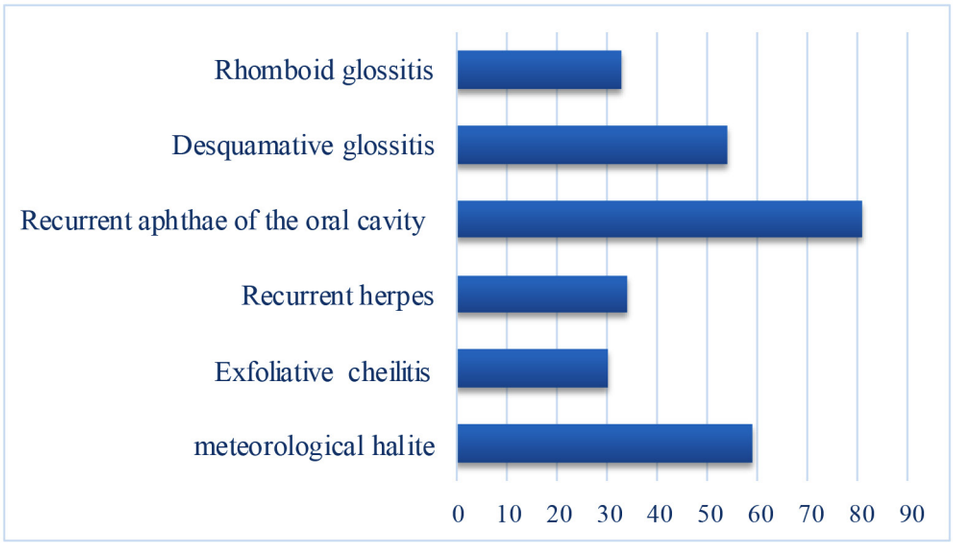
Education "Bashkir State Medical University" in Ufa, and 90 men and women aged 24 to 45, with chronic gastritis and duodenitis (49 patients), chronic pancreatitischole, cholecystitis and biliary dyskinesia (51 patients) were selected in a targeted sample. The study of the state of the oral microbiome in individuals with recurrent aphthous stomatitis made it possible to compare the groups of microorganisms according to the degree of their prevalence, as well as to identify those that are of the greatest importance in the formation of the microecosystem.

During the comprehensive dental examination of patients with gastrointestinal diseases, recurrent oral aphthae were diagnosed in an average of 37% of the patients examined. Painful elongated aphthae with a circle of hyperaemia and covered with dense yellowish fibrinous plaque that cannot be removed during scraping were more often located on the mucous membrane of the transitional fold of the upper lower jaw in 28,9% of cases; more rounded aphthae were located on the transitional fold of the mucous membrane of the upper or lower lips in 21,1%, on the lateral surface or on the tip of the tongue - in 18,9%, on the mucous membrane of cheeks - in 5,5% and a mixed form - in 25,6% of the patients examined. The mucous membrane of the mouth is brighter in colour, the aphthae are oval in shape, up to 3 mm in size, with clear boundaries and a rim of hyperaemia, covered with fibrinous pellicle, with sharp pain on palpation. The duration of aphthae occurrence is no more than 10-14 days, the periods of remission are also different and can vary on average from 1-2 to 6-12 months. During the healing period of aphthae on the mucous membrane, a whitish, opalescent area remains at the place of their location, most often disappearing without a trace within 7-10 days, which indicates a fibrinous form according to the ICD-10 Classification — small aphthae of moderate severity. However, when interviewing patients, the most painful single or multiple aphthae were located in transitional folds and on the lateral surface of the tongue. In the oral cavity, this was clinically apparent in increased tissue infiltration, hyperaemia in the area of aphthae location.

When examining the organs of the oral cavity, the state of a red border of lips and corners of the mouth were studied. Attention was given to their colour, size and the presence of pathological elements. This category of patients most often complained of dryness and unaesthetic appearance. 58,8±0,33% of the examined were diagnosed with meteorological cheilitis (dryness of vermilion, scaly crusts and cracks), 29,6±0,66% - exfoliative cheilitis (dry form) - greyish scaly crusts, tightly attached to the vermilion in the centre and falling off along the edges, easily removable, with exposure of a bright red surface. In the clinical dental examination, only 11,7±0,15% of patients had vermilion without pathological changes. Recurrent herpes was detected on average in 33,8 ± 0,08% of cases. Desquamative glossitis was diagnosed in 54,4±1,4%, rhomboid glossitis - in 33±1,2% of the examined (Figure 1).

In patients with chronic gastritis, the general condition is not disturbed, the skin is clean, regional lymph nodes are not palpated, the face configuration is not changed. Clinical manifestations of recurrent oral aphthae on the oral mucosa are characterized by the appearance of painful oval secondary elements - aphthae with a size of 0.1 to 0.8 mm, covered with fibrinous yellowish-white pellicle in an average amount of 1 to 3, around the periphery the aphthae are surrounded with a circle of hyperaemia or inflammatory infiltration is observed. The main complaints received during the survey of patients with chronic gastritis and duodenitis are severity and pain in

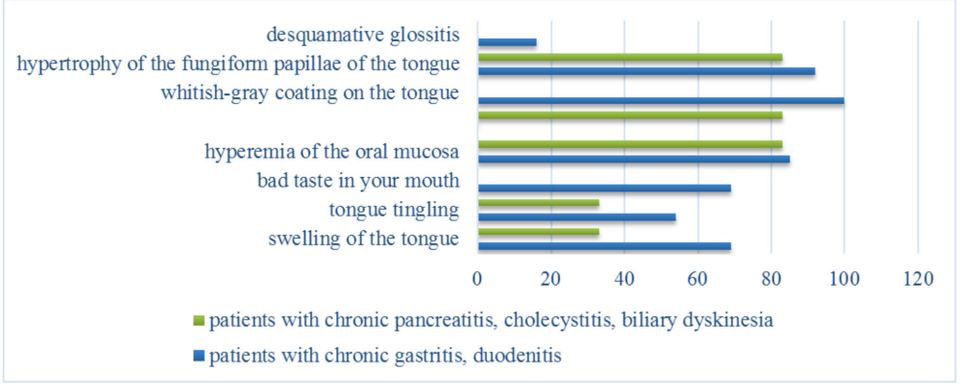
Figure 1. The structure of pathology of the mucous membrane in young people with gastrointestinal diseases



the epigastric region, impaired taste perception in 69% of cases, respectively. In the oral cavity, the mucous membrane is hyperaemic at the place of aphthae location in 37% of cases, whitish-grey plaque was observed on the dorsal surface of the tongue - in 100%, hypertrophic changes in tongue clavate papillae - in 92% and desquamative glossitis - in 16,2% of cases. In patients with chronic duodenitis, chronic pancreatitis and cholecystitis with biliary dyskinesia, the general condition is satisfactory. Patients most often complain of burning and soreness in the tongue, aggravated in the evening, or bitterness in the mouth - 33% of cases. An atrophy of filiform papillae and yellow plaque on the dorsal surface of the tongue, hyperaemic mucosa of the mouth vestibule and the oral cavity are objectively determined in 83% of cases, tongue edema, dryness and taste disturbance are observed on average in 33% of the examined (Figure 2).

In patients with chronic recurrent aphthae, representatives of groups Enterococcus spp., Staphylococcus spp., Streptococcus spp. are detected in 100% of cases. In 45,6% of cases, microorganisms not characteristic of the oral cavity,

Figure 2. The main comxplaints of patients with chronic pancreatitis, cholecystitis and biliary dyskinesia



both of aerobic and anaerobic types of respiration - Escherichia spp., Klebsiella spp., Bacteroides spp., are revealed in the examined patients (Figure 3).

In 20% of cases, the content of yeast-like fungi of the genus Candida (C. albicans) in 10^1 - 10^2 CFU/ml was detected in patients with recurrent aphthae, in 39.2% of cases a quantitative increase in yeast-like fungi of the genus Candida (C. albicans) from 10^3 to 10^4 CFU/ml was observed, in 40.8% of cases - from 10^3 to 10^5 CFU/ml ($p \leq 0.05$). The number of yeast-like fungi in the oral cavity among the young patients examined with recurrent oral aphthae ranged from l g 1.0 CFU/ml to l g 5.0 CFU/ml.

Microscopic examination of smears from various parts of the oral cavity has revealed that yeast-like fungi of the genus Candida (C. albicans) are most often found in tongue scrapings in 55% of cases, cheek and gum mucosa in an average of 22% of cases (Figure 4).

Figure 3. The composition of the oral microbiome with recurrent oral aphthae

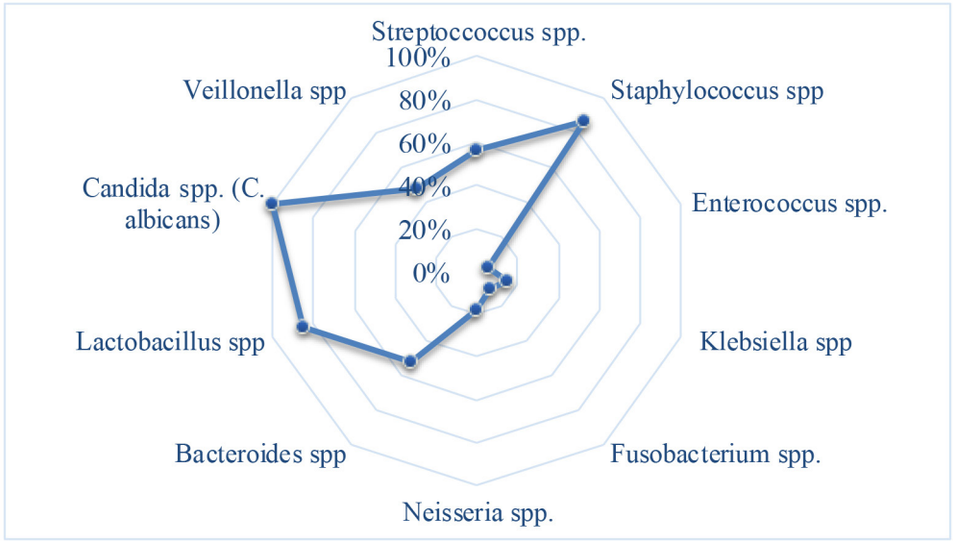
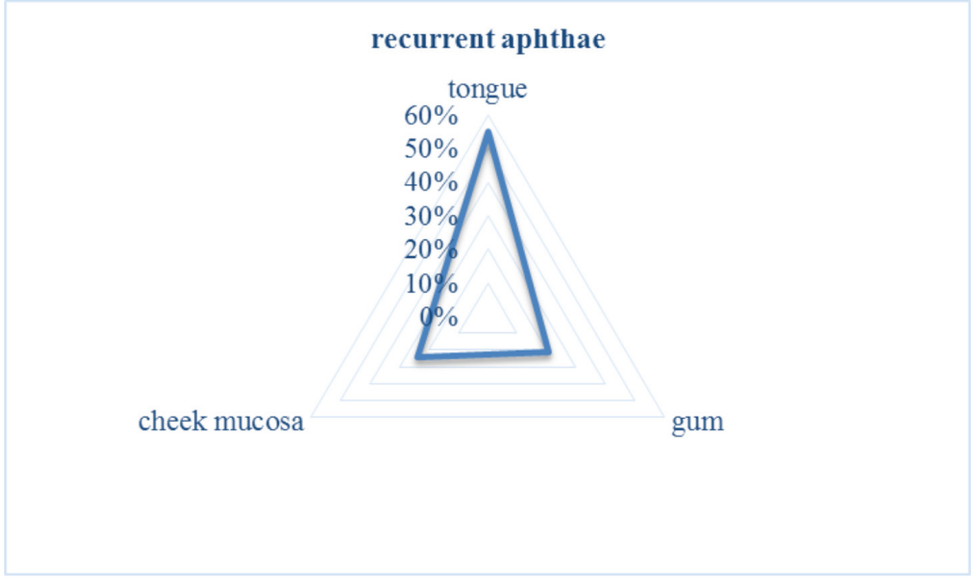


Figure 4. The amount of C. albicans in the oral microbiome with recurrent aphthae



According to the results of PCR diagnostics, in patients with recurrent oral aphthae the average prevalence of obligate-anaerobic microorganisms in the affected areas - Porphyromonas gingivalis, Treponema denticola, Aggregatibacter actinomycetemcomitans, Tannerella forsythensis, Fusobacterium nucleatum, Prevotella intermedia is observed in 64% of cases.

Conclusions

Recurrent oral aphthae are detected in 36% of the patients with diagnosed general somatic pathology.

Identification of individual features of the oral microbiome in chronic recurrent oral aphthae includes a combination of microbiological studies and PCR diagnostics.

The results of the in-depth study of the oral microbiome showed frequent detection of bacteria of the genus Enterococci, streptococci and yeast-like fungi of the genus Candida (C. Albicans), Porphyromonas gingivalis, Treponema denticola, Aggregatibacter actinomycetemcomitans, Tannerella forsythensis, Fusobacterium nucleatum, Prevotella intermedia.

Thus, patients with recurrent oral aphthae have the violated natural microbiome of the oral cavity, which is of great prognostic value for the development of effective therapeutic and preventive measures in this category of patients.

References

1. Bulkina, N.V., Meleshina, O.V., Lomakina, D.O. Chronic recurrent aphthous stomatitis: clinical course and complex treatment. *Saratov Journal of Medical Scientific Research*, volume 7(1), pp. 281-282, 2011.
2. Gazhva, S.I., Leskov, A.S., Shkarednaya, O.V. et al. Features of the dental status of patients with gastroduodenal pathology. *Review*, volume 1(75), pp. 4950, 2012.
3. Karakov, K.G., Vlasova, T.N., Sirak, S.V., Porfiriadis, M.P., Lavrinenko, V.I., Oganyan, A.V., Khachaturian, E.E., Sarkisov, A.A., Khubaev, S-S.Z., Mordasov, N.A. Recurrent and scarring aphthae in case of gastrointestinal diseases. *Scientific Review. Abstract journal*, volume 5, pp. 19-20, 2016.
4. Makeeva, I.M., Martynova, E.A., Rozhnova, E.V. Oral cavity as a local ecological system. *Dentistry*, volume 3, pp. 68-75, 2008.
5. Orekhova, L.Yu., Kudryavtseva, T.V., Cheminava, N.R., Tachalov, V.V., Loboda, E.S. Dental health issues in young people (literature review). *Periodontology*, volume 2(71), pp. 3-5, 2014.
6. Rabinovich, O.F., Rabinovich, I.M. Recurrent aphthous stomatitis: classification, clinical forms and treatment. *Dentistry*, volume 78(3), pp. 76-79, 2010.
7. Round, J.L., Mazmanian, S.K. The gut microbiota shapes intestinal immune responses during health and disease. *Nat. Rev. Immunol*, volume 9, pp. 313–323, 2009.
8. Clemente, J.C., Ursell, L.K., Parfrey, L.W., Knight, R. The Impact of the gut microbiota on human health. An Integrative View. *Cell.*, volume 148, pp. 1258–1270, 2012.
9. Petrukhina, N.B., Zorina, O.A., Shikh, E.V., Shibaeva, A.V., Shevelev, A.B. Study of mutual dependence of periodontal and intestinal microbiome in health and pathology using deep-sequencing methods. *Dentistry*, volume 95(2), pp. 8–1332, 2016
10. Usmanova, I.N., Tuigunov, M.M., Gerasimova, L.P., Kabirova, M.F., Gubaidullin, A.G., Gerasimova, A.A., Khusnarizanova, R.F. Role of opportunistic and pathogenic microflora of the oral cavity in the devel-

opment of inflammatory diseases of periodontal and oral mucosa (literature review*). Bulletin of the South Ural State University. Series: Education, Healthcare, Physical Education*, volume 15(2), pp. 37-44, 2015.
11. Usmanova, I.N. Features of microbiocenosis of the oral cavity in young people living in a region with adverse environmental factors. *Clinical Dentistry*, volume 3, pp. 94-96, 2011.
12. Shibaeva, A.V., Aivazova, R.A., Rebrikov, D.V., Trubnikova, E.V., Kudykina, Yu.K., Belyakova, A.V. et al. Use of the real-time PCR method for studying the periodontal microbiome in patients with combined pathology of gastroduodenal zone and chronic periodontitis. *Molecular Genetics, Microbiology and Virology*, volume 34(1), pp. 26-30, 2016.
13. Jünemann S., Prior K., Szczepanowski R., Harks I., Ehmke B., Goesmann A., et al. Bacterial community shift in treated periodontitis patients revealed by ion torrent 16S rRNA gene amplicon sequencing. *PloS One*, volume 7(8), e41606, 2012.