

BACTERIOLOGICAL STUDY OF ORAL MICROBIOCENOSIS AS A STARTING POINT FOR BEGIN PHARMACEUTICAL TREATMENT

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Abstract

Changes in the anatomy of the marginal gums inevitably affect the course of physiological processes in the oral cavity and the nature of the oral microbiocenosis. It is the microbial factor that plays an initiating role in the occurrence of the inflammatory process in periodontal and gum tissues, contributes to its chronicity, causes local manifestations of intoxication and sensitization. Therefore, a quality preparation of the tooth stump with the missing crown part for orthopaedic reconstruction shall take into account this pathogenetic mechanism of damage to the tissues of the gums and periodontium. We have examined 142 patients, including 112 patients with defects of hard tissues of the frontal group of teeth, destroyed below the level of the gums due to the carious process and untimely treatment. The control group consisted of 30 people with preserved dentition and without periodontal pathology. Compared with healthy individuals in the control group, all of the examined patients turned to have significant qualitative and quantitative changes in the characteristics of the microbiocenosis of the oral cavity in the stump of the tooth root with the missing crown. Only 6 of 42 patients with defects of the coronal part of the teeth had the total number of microorganisms in the gingival fluid within the confidence interval of the corresponding indicator error, typical for the control group. 12 patients in this group had a detected total number of microorganisms, almost two orders of magnitude (more than 100 times) higher than the control level. It should be noted that the constancy index of the resident microflora of the oral cavity, which reflects the frequency of detection of this microorganism in the examined group of patients, in the process of using different methods of preparation of the gingival margin underwent minimal changes. Among the dominant participant, oral microbiocenoses in the gingival fluid in the stump of the tooth root with the missing coronal part is more significant than in the gingival fluid from the gingival sulcus of intact teeth, β -haemolytic streptococci (*Streptococcus pyogenes*, *Streptococcus constellatus*, *Streptococcus* group G), *Veillonellaparvula*, *S. aureus*, micrococci, diphtheroids and yeast-like fungi of the genus *Candida*. At the same time, in the structure of microbiocenoses formed in the area of the root stump, a decrease in the proportion of stomatococci, *S. epidermidis* and bacilli is observed. Therefore, quality preparation of the tooth stump with the missing crown for orthopaedic reconstruction shall take into account this pathogenetic mechanism of gum and periodontal tissue damage to prevent the development of complications of microbial origin after orthopaedic treatment.

Key words: *microbiocenosis, the area of the stump of the tooth root, gingival fluid, pathogenic microflora.*

Introduction

After the loss of the tooth crown and against the background of deep subgingival root destruction, the natural line of the marginal gums is deformed due to the hypertrophic growth of the marginal epithelium and granulation tissue [3]. Changes in the anatomy of the marginal gums inevitably affect the course of physiological processes in the oral cavity and the nature of the oral microbiocenosis. Hypertrophied gingival margin prevents full drainage of the surface formed in the area of the tooth root, contributes to the retention of plaque and oral fluid in it, and creates ideal conditions for the development of microflora. Thus, a special ecological niche is formed in the area of the tooth root. It is the microbial factor that plays an initiating role in the occurrence of the inflammatory process in the tissues of the periodontium and gums, contributes to its chronicity, causes local manifestations of intoxication and sensitization [2].

Despite the large number of studies examining the microbiocenosis of the oral cavity of patients with various dental pathologies and users of removable dentures, the nature and characteristics of the microflora in the area of the tooth root with the lost crown are insufficiently clarified. In this regard, studies that demonstrate the similarity of the microflora of the root canals and adjacent periodontal pockets in combined periodontal-endodontic lesions of the teeth with both intact and affected coronal part are interesting [12, 13]. Obligatory-anaerobic bacteria *Eikenella corrodens*, *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Treponema denticola*, including species that are not usually associated with periodontal-endodontic affection (*Lactococcus lactis*, *Peptostreptococcus micros*, *Haemophilus influenzae*) were isolated from the root canal [6, 9, 11]. Facultative anaerobic α -haemolytic streptococci are present in 100% of the studied samples from the tooth root canal [4, 21].

Therefore, a quality preparation of the tooth stump with the missing crown part for orthopaedic reconstruction must take into account this pathogenetic mechanism of damage to the tissues of the gums and periodontium. The procedure of forming a new relief of the gums should eliminate

the anatomical preconditions that contribute to the violation of the oral microbiocenosis, and at the same time prevent the development of complications of microbial origin after orthopaedic treatment [17]. In addition, the correction of the marginal gums is important for taking a full imprint and making a quality orthopaedic structure (single crown or bridge). After all, ensuring a perfect edge fit of the tab and the crown frame to the ledge is one of the determining factors in the duration of the patient's use of the orthopaedic design.

The literature describes several ways to correct the marginal gums around the tooth [5]. An important problem of dentistry today is the treatment of teeth destroyed below the free part of the gums, and the condition of the periodontium [3]. The condition of the epithelial plate of the gingival margin is important for the choice of treatment, because the epithelium provides mainly homeostasis of the oral cavity [8, 10].

Methods

The study was approved by the Ethics Committee of the Faculty of Dentistry of Ivano-Frankivsk National Medical University. Clinical examination of patients was performed on the basis of the clinic of the Department of Dentistry of the Research and Training Institute of Postgraduate Education of Ivano-Frankivsk National Medical University. Microbiological research methods were conducted on the basis of the Department of Microbiology, Virology, Immunology of IFNMU.

142 patients aged 25-44 were examined, including 112 patients with defects of hard tissues of the frontal group of teeth, destroyed below the level of the gums due to the carious process and untimely treatment of dental care. The control group consisted of 30 people with preserved dentition and without periodontal pathology, who were admitted in one visit during which they took material for microbiological examination to compare the data of the individuals of main groups.

Bacteriological examination of the oral microflora is necessary for effective and controlled removal of the layer of hypertrophied tissues around the tooth root and reduction of the duration of orthopaedic treatment.

Material for bacteriological examination to detect aerobic and facultative anaerobic microflora, as well as yeast-like fungi were taken from the stump of the tooth root destroyed below the gingival margin, which was subject to orthopaedic treatment by making solid stump inserts. The gingival fluid was collected with a calibrated bacteriological loop and immediately seeded on blood agar. For the patients with defects of hard tissues of teeth destroyed below the level of the gums, microbiological examination of gingival fluid was performed twice - before preparing the stump of the tooth for orthopaedic treatment and after applying various methods of forming new gum relief: excision of the hypertrophied edge of the epithelium of the cervical region with a microsurgical scalpel, electrocoagulation and laser coagulation. To establish the parameters of the normal microbiocenosis of the oral cavity of healthy individuals (control group), a similar way was used for withdrawal of gingival fluid.

Showings were performed by the Gold method, which allows a quantitative assessment of the level of microbial seeding. The seeds were incubated for 1 day at 37 °C under aerobic and anaerobic conditions (in a hermetically sealed desiccator) in an atmosphere enriched with carbon dioxide. Identification of isolated pure cultures was performed by a set of morphological, cultural and biochemical properties (sets "STREPTOtest 16", "STAPHYtest 16", "ENTEROtest 24", Lachema, Czech Republic). Particular attention was paid to the nature of hemolysis, plasma coagulase and lecithinase activity of staphylococci, lactase activity of enterobacteria, pigmentation.

The bacteriological study of crop took into account the presence of the following microorganisms: 1) -hemolytic *Streptococcus pyogenes*, 3) *Staphylococcus aureus*, 4) *Staphylococcus epidermidis*, 5) *Stomatococcus mucilaginosus*, 6) *Veillonella parvula*, 7) *Micrococcus* sp., 8) *Corynebacterium* sp., 9) *Bacillus* sp., 10) *E. Coli* and other species of enterobacteria, 11) yeast-like fungi *Candida* sp.

Quantitative accounting of colonies was carried out taking into account their species (or family)

affiliation. Based on the analysis of crop results for microorganisms in each group, the constancy index, population level (expressed in lg CFU/ml) and the coefficient of quantitative dominance were determined, which allowed to judge the nature of the microbiocenosis in the area of the stump of the tooth root, destroyed below the gingival margin, as well as to monitor its changes in the process of applying various methods of forming a new relief of the gums.

Statistical processing of the obtained results was carried out using a personal computer and licensed applications for working with Microsoft Excel spreadsheets and the package "STATISTICA 7.0".

Results

The absence of the tooth crown leads to the formation of a special ecological niche. Its existence is supported by a hypertrophied gingival margin, which prevents full drainage of the formed cavity, contributes to the retention of food residues and oral fluid. This creates ideal conditions for the development of microflora. Our microbiological studies of gingival fluid collected in the stump of the tooth root show that the total number of microorganisms in it is significantly higher (6.42 ± 0.15 lg CFU / ml, $p < 0.05$) than in gingival fluid collected in area of the gingival sulcus of patients with intact dentitions (4.94 ± 0.25 lg CFU / ml).

Only 6 of 42 patients with defects of the coronal part of the teeth ($14.3 \pm 5.40\%$) had the total number of microorganisms in the gingival fluid within the confidence interval of the corresponding indicator error ($M \pm 2\sigma$), typical for the control group. 12 patients of this group $28.6 \pm 6.97\%$ were detected the total number of microorganisms, almost 2 orders of magnitude (more than 100 times) higher than the control level.

To study the nature of the oral microbiota in the area of the stump of the tooth root, we divided the microorganisms found in gingival fluid into 4 groups: 1) representatives of the resident microflora of the mouth (-haemolytic streptococci – *Streptococcus alivarius*, *Streptococcus mitis*, *Streptococcus anguis*, *Stomatococcus mucilaginosus*, *Veillonella parvula*; 2) representatives of the transient pathogenic microflora (*Staphylococcus epidermidis*,

corynebacteria *Corynebacterium xerosis*, *Corynebacterium flavescens*, bacilli, micrococci); 3) pathogens and opportunistic microorganisms with high pathogenic potential, characteristic of this biotope (β -haemolytic streptococci, *Staphylococcus aureus*, yeast-like fungi of the genus *Candida*) and 4) opportunistic pathogens, not characteristic of this biotope (*Escherichia coli* and other enterobacteria, pseudomonads). For all microorganisms, the population level (PL) was determined - the massiveness of colonization of this biotope (which was expressed in lg CFU / ml) and the constancy index (CI) - the frequency of detection of this microorganism in the examined group of patients (%). A more holistic view of the nature of the microbiocenosis is given by the coefficient of quantitative dominance (CQD) - the percentage of this microbe among all detected microbial cells in a particular patient.

Almost all examined patients observed significant qualitative and quantitative changes in the characteristics of the microbiocenosis of the oral cavity in the stump of the tooth root with the missing crown, compared with healthy individuals in the control group (Table 1).

The main representatives of the oral microflora - α -haemolytic streptococci were found in all samples of oral fluid collected in the stump of the root of the destroyed tooth, as well as among all healthy individuals (CI 100.0%). However, the population level of α -haemolytic streptococci in the stump of the tooth root is significantly higher 6.26 ± 0.16 lg CFU/ml) than among healthy individuals of the control group (4.81 ± 0.27 lg CFU / ml, $p < 0, 01$). Another representative of the resident microflora of the oral cavity *Veillonellaparvula* was isolated in the stump of the root almost three times more often ($57.1 \pm 1.2\%$ vs. $18.8 \pm 2.4\%$, $p < 0.01$) and an order of magnitude more (4.32 ± 0.19 lg CFU / ml vs. 3.57 ± 0.38 lg CFU / ml, $p < 0.05$) than in the control. This may be evidence of the intensive formation of anaerobiosis zones in this ecological niche. In terms of frequency of detection (CI) and mass colonization (PL) of *Stomatococcus mucilaginosus*, the two groups are almost indistinguishable.

The massive colonization of the oral cavity in the stump of the roots of the teeth with a defect of the coronal part of the transient oral non-pathogenic microflora (*Staphylococcus epidermidis*, corynebacteria, bacilli, micrococci) also shows a clear tendency to increase. Corynebacteria are present in the area of the root stump significantly more often than in the area of the gingival sulcus of healthy individuals (IP $16.7 \pm 0.9\%$, $p < 0.05$). However, the bacillary microflora in the area of the root stump is, on the contrary, less common than in the gingival fluid of the control group.

Pathogenic coccal microflora (β -haemolytic streptococci *Streptococcus pyogenes*, *Streptococcus constellatus*, *Streptococcus gordonii*, *Streptococcus groupG* and *Staphylococcus aureus*) in the gingival fluid collected in the stump of the roots of the teeth with a defect of the crown, is present significantly more often than among healthy individuals of the control group: CI for *S. Aureus* is 2.1 times, and β -haemolytic streptococci - 9.5 times higher than the corresponding control values ($p < 0,05$). However, the population level of *Staphylococcus aureus* is approximately the same as in the gingival fluid from the gingival sulcus of intact teeth. β -haemolytic streptococcus *S. pyogenes* was detected in the gingival fluid of only one person from the control group, although in a sufficiently high amount - 4.70 ± 0.01 lg CFU / ml. The population level of β -haemolytic streptococci in the area of the root stump is slightly lower - 3.68 ± 0.15 lg CFU / ml. But β -haemolytic streptococci were isolated in 25 of 42 samples of gingival fluid from the stump of the teeth roots ($59.5 \pm 1.2\%$, $p < 0.001$).

In addition to pathogenic coccal microflora from the gingival fluid collected in the stump of the teeth roots with a defect of the coronal part, quite often (CI $11.9 \pm 0.8\%$) yeast fungi of the genus *Candida* were sown, with an average population colonization level of 3.30 ± 0.14 lg CFU / ml. At the same time, none of the examined yeast-like fungi were not detected in the oral fluid from the gingival sulcus of intact teeth.

The presence of enterobacteria and *Pseudomonas aeruginosa* in the oral cavity is not typical for the control group of healthy individuals. *Escherichia coli* was detected in gingival fluid of only

2 patients ($4.8 \pm 0.5\%$) with defects in the crown of the teeth (with a low level of colonization – PL 2.70 ± 0.01 lg CFU / ml). *Pseudomonasaeruginosa* in none of the studied gingival fluid samples was found by us at all.

If we take into account the total number of microbial cells, it is seen that in the gingival fluid both from the stump of the root and from the gingival sulcus of intact teeth, α -haemolytic streptococci undoubtedly dominate. The share of the examined healthy individuals averaged $79.75 \pm 6.53\%$. The proportion of α -haemolytic streptococci in the gingival fluid collected in the stump of the tooth root with the missing coronal part is slightly higher – $84.95 \pm 4.05\%$ ($p > 0.05$). In addition, the normal microbiocenosis of the gingival sulcus of intact teeth of our healthy individuals was characterized by high efficiency ($35.83 \pm 4.14\%$) of epidermal staphylococcus *S. epidermidis*.

Among the minor participants of oral microbiocenoses in the gingival fluid in the area of the stump of the tooth root with the missing coronal part β -haemolytic streptococci (*Streptococcus pyogenes*, *Streptococcus constellatus*, *Streptococcus group G*), *Veillonella parvula*, *S. aureus*, micrococci, diphtheroids and yeast-like fungi of the genus *Candida* (Figure 1) are present significantly more often than in the gingival fluid from the gingival sulcus of intact teeth.

At the same time there is a decrease in the proportion of stomatococci (from $14.48 \pm 6.41\%$ to $1.02 \pm 0.37\%$, $p < 0.01$), *S. epidermidis* (from $35, 83 \pm 4.14\%$ to $1.89 \pm 0.41\%$, $p < 0.05$) and bacilli (from $0.11 \pm 0.03\%$ to $0.029 \pm 0.004\%$, $p < 0.05$) in the structure of microbiocenoses formed in the area of the root stump.

Discussion

Therefore, high-quality preparation of the stump of the tooth with the missing crown part for orthopaedic reconstruction must take into account this pathogenetic mechanism of gum and periodontal tissue damage. The procedure of forming a new relief of the gums should ensure the elimination of anatomical preconditions that provoke a violation of the oral microbiocenosis, and at the same time prevent the development of

complications of microbial origin after orthopaedic treatment.

During the treatment of periodontal disease, positive results can be achieved by combining different methods of treatment, both therapeutic, surgical, medical and physiotherapeutic. Local treatment of periodontal disease requires prolonged contact of the medication with the surrounding dental tissues [5, 6]. Physiotherapeutic methods of treatment should be used to prolong the effect of drugs and to avoid additional drug load on patients with periodontal disease [7]. A feature of physiotherapy procedures is a gradual, profound effect on the tissues, which gives long-term positive results in all forms and stages of periodontal disease. Acting on neurohumoral and reflex mechanisms, physical therapies stimulate redox processes in the periodontium, restore metabolism, improve tissue trophism and metabolism. As a result, they improve blood and lymph circulation, inhibit the growth of granulation tissue, reduce inflammation and congestion. Physical factors increase the activity of connective tissue elements, phagocytic activity of leukocytes, and, consequently, increase local immunity, accelerate the regeneration process [8, 9]. Physiotherapy is used in almost all forms and severity of periodontal disease as pathogenetic and symptomatic therapy in complex treatment, prevention and rehabilitation.

The analysis of 10 most important basic life support systems of human body — cardiovascular (CVS), respiratory (RS), nervous (NS), digestive (DS), endocrine (ES), immune (IS), excretory (EXS), brain (BS), musculo-skeletal (MSS), hematopoietic (HS) was carried out. Based on this analysis two levels of ensuring the reliability of organism's work were revealed: sequential and parallel. The system of logical equations for reduced sequential system is: $Ys_1 = CVS \ RS \ BS$, where is the notation for the conjunctions of set elements. The system of logical equations for the reduced parallel system is: $Ys_2 = NS \ DS \ ES \ IS \ HS \ EXS \ MSS$, where is the disjunction of the scheme elements. Visualization of human STC changes the concept of the kinetics of age-related changes in the organism and the role of determinants of health as a stable factor accompanying a uniform, smooth transition from the most pronounced functions of the body to their

gradual extinction. For human STC is formulated the following regularity kinetics of involutory processes: after 30 years of age in the human body morphological changes regress in arithmetic progression, and the functions of organs in a geometric one. Assumption of health as a state redundancy of functions is suggested [22].

The research is devoted to the fundamental issue of medicine and biology – the study of factors limiting the life span of a person. As a model, the system of adaptation of the human body to the forces of natural gravity is chosen, the disadaptation to which manifests itself in falls and everyday injuries. The object of the study was the selection of severe fractures of bone tissue due to fall, taken in the age aspect. Statistical and meta-analytical research methods were used. It is shown that the age-related increase in mortality due to household falls, coming to severe bone fractures, is non-linear and increases in geometric progression. As a result of the coincidence of the age characteristics of bone fragility and age-related kidney function, an assumption is made about the role of involution of the renal tissue in the development of osteoporosis in the elderly and the need for a new approach to the prevention of osteoporosis and domestic injuries [23].

Conclusions

Thus, the analysis of the results of bacteriological examination shows that in the absence of the crown of the tooth with the participation of hypertrophied gingival margin, a special pathogenic microflora is formed. It is characterized by a significant increase in the total number of symbiont microorganisms, more intensive colonization of the habitat by optional and obligate anaerobic bacteria – α -haemolytic streptococci, veilonella, micrococci and diphtheroids against the background of a decrease in the proportion of stamococcus and epidermal staphylococci. Pathogenic coccal microflora (β -haemolytic streptococci, *Staphylococcus aureus*), as well as yeast-like fungi of the genus *Candida* are significantly more frequent and present in the gingival fluid collected in the stump area of tooth roots with a defect of the coronal part. In some cases, enterobacteria (*E. coli*), which are not peculiar to the normal microbiocenosis of the oral cavity, were found in the stump area of the tooth

roots. Detected violations of the microecology of the oral cavity of patients with defects of the hard tissues of the teeth destroyed below the gingival margin should be taken into account when carrying out orthopaedic reconstruction.

Compliance with Ethical Standards

Conflict of Interest. The authors declare that there is no conflict of interest that could be perceived as interfering with publication of the article.

Competing Interests. The authors declare that they have no competing interests.

Ethical Approval. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent. Informed consent was obtained from all individual participants included in the study. All subjects of the institutional survey gave consent for anonymized data to be used for publication purposes.

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Table 1. Characteristics of oral microbiocenosis in the stump of the tooth root, destroyed below the gingival margin

Groups of microorganisms	Patients with missing coronal part (n=42)			Control (n=16)		
	CI	PL	QDC	CI	PL	QDC
α -haemolytic <i>Streptococcus sp.</i>	100,0±0,01	6,26±0,16*	84,95±4,05	100,0±0,01	4,81±0,27	79,75±6,53
β - haemolytic <i>Streptococcus pyogenes</i>	59,5±1,2*	3,68±0,15*	3,22±1,00*	6,3±1,5	4,70±0,01	0,99±0,01
<i>Staphylococcus aureus</i>	26,2±1,1*	3,59±0,15	7,09±2,21*	12,5±2,1	3,35±0,12	2,18±0,77
<i>Staphylococcus epidermidis</i>	23,8±1,0*	4,68±0,11*	1,89±0,41*	31,2±2,9	3,62±0,09	35,83±4,14
<i>Stomatococcus mucilaginosus</i>	61,9±1,2	3,86±0,14	1,02±0,37*	56,3±3,1	3,49±0,20	14,48±6,41
<i>Veillonella parvula</i>	57,1±1,2*	4,32±0,19*	13,34±4,61*	18,8±2,4	3,57±0,38	2,90±1,05
<i>Micrococcus luteus</i>	2,4±0,4*	6,70±0,10*	4,54±1,56*	0	0	0
<i>Corynebacterium sp.</i>	16,7±0,9*	4,21±0,279	14,65±4,76*	6,5±1,5	3,00±0,03	0,20±0,005
<i>Bacillus sp.</i>	4,8±0,5*	3,70±0,22*	0,029±0,004*	12,5±2,1	2,00±0,01	0,11±0,03
<i>E. coli</i>	4,8±0,5*	2,70±0,01*	0,025±0,005*	0	0	0
<i>Candida sp.</i>	11,9±0,8*	3,30±0,14*	0,14±0,03*	0	0	0

Notes:

1. CI – constancy index (%), PL – population level (lg CFU/ml), QDC – quantitative dominance coefficient (%).
2. * - p < 0.05 when compared with the control.

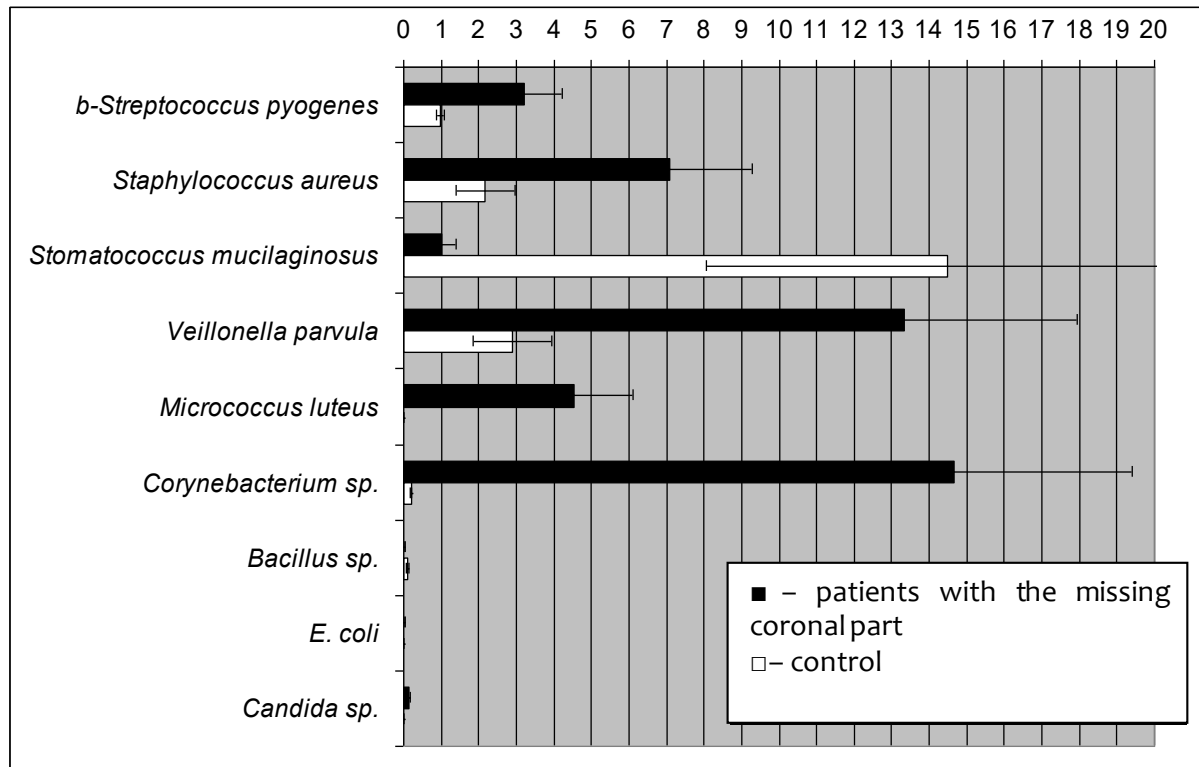


Figure 1. Coefficient of quantitative dominance (in%) of minor participants of oral microbiocenoses in gingival fluid of patients with defects of hard tissues of teeth destroyed below the gingival margin and healthy individuals.