ORIGINAL PAPER

COLONIZATION RESISTANCE OF ORAL MUCOSA IN INDIVIDUALS WITH DIVERSE BODY MASS INDEX

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ABSTRACT

Introduction: Colonization resistance of the oral cavity is an important protective factor of local immunity, which prevents the adhesion and proliferation of microorganisms on the oral mucosa.

Objectives: The research aimed to discover the colonization resistance state of the oral mucosa of young patients with different body mass indices and their dependence on the intensity of the teeth carious and inflammatory response of the gums.

Material and methods: The survey conducted on 132 Ukrainians of all genders, with aged ranging from 18 to 22 years. Body mass index (BMI) was determined, which was the distribution factor. Oral status was detected by decay, missing, and filled teeth (DMFT) index, oral hygiene index (OHI) and papilla bleeding index (PBI) determination. Colonization resistance of the oral mucosa was defined by microscopy of the buccal epithelium using a light microscope with a magnification of ×400.

Results: 21.2% of patients with normal BMI had colonization resistance index (CRI) 0, 78.8% – CRI 1. In patients with extra weight, 44.5% had CRI 0, 38.9% – CRI 1, 16.6 – CRI 2. In patients with 1st degree obesity, 64.5% had CRI 0, 25.8% – CRI 1, 9.7% – CRI 2. In patients with 2nd degree obesity, 68.75% presented CRI 0, 31.25% – CRI 1.

Conclusions: With satisfactory oral hygiene in patients with diverse BMI the severity of gingivitis was different. This indicates that the systemic response of the organism in patients with BMI over 30 kg/m^2 is the crucial determining factor that influences the manifestation of the disease, as a response to local pathogenic factor – dental plaque. Therefore, in patients with 1st and 2nd degree obesity, in 70% of patients, suppression of colonization resistance of the oral mucosa was observed, compared with patients with normal BMI where the frequency of patients with colonization resistance disturbance was 2.5 times lower.

Key words: obesity, periodontitis, gingivitis, colonization resistance.

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INTRODUCTION

Obesity is a widespread health issue that has a high prevalence in all age groups worldwide. Extra weight and obesity are risk factors for diverse oral cavity diseases, such as periodontal conditions and oral chronic infectious disease. Although, obesity increases susceptibility to bacterial infection. The precise pathophysiological mechanisms that link obesity and susceptibility to periodontal inflammatory and non-inflammatory diseases,

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remain undiscovered in depth. Obesity increases the risk of periodontal disease by raising production of uric acid mediated by gut dysbiosis [1].

Disturbance of the microbial homeostasis in oral cavity is the cause of periodontal diseases and tooth hard tissue lesions. There are several hypotheses on the development of periodontal disease caused by dental plaque. Non-specific plaque hypothesis suggests that the total bulk of plaque has a more harmful effect rather than the presence of particular pathogenic microbial species. Specific plaque hypothesis implies that the particular bacteria in dental plaque play a crucial role in the development of disease. A third concept is multiple pathogen theory that implicates combinations of specific microorganisms. Manifestation of a disease is triggered by a misbalance between the host and pathogenic/opportunistic microorganisms [2].

It is necessary to assess the level of colonization resistance of the oral mucosa, which reflects the organism response to microbial factors and understand adaptive mechanisms of local immunity to predict the occurrence of disease. It makes it possible to predict and prevent the development of a disease even before its' manifestation. Moreover, functional alterations in periodontal tissues lead to the development of clinical changes [3].

Wide spectrum of oral cavity microbes and their colonization is mostly based on commensalism. They are part of the normal oral microflora, but there are also opportunistic species that can cause oral and systemic diseases, when protective mechanisms of an organism are affected by some systemic conditions. In some circumstances, the imbalance between normal oral flora and pathogenic flora may change the ratio of commensalism to parasitism. Oral mucosa is impermeable to most microorganisms and constitutes a mechanical barrier against their penetration into tissues [4].

Microbiome of oral cavity in obese individuals has specific quantitative and qualitative content. Analysis of oral cavity microbiota showed a high content of *Capnocytophaga* genus in obese individuals compared with patients with normal body mass index. Females with extra weight had an increase in *Streptococcus* subspecie content compared with obese males, where *Neisseria* genus was in abundance [5].

Obese individuals have completely different microbiological content. *Negativicutes*, *Erysipelotrichia*, *Prevotella melaninogenica*, *Prevotella salivae*, *Solobacterium moorei*, and *Atopobium parvulum* were detected in higher quantity in obese individuals, whereas *Flavobacteriia*, *Bateroidetes*, and *Streptococcus sanguinis* were predominant in individuals with normal BMI. The level of species showed significant differences in relative abundance among the groups, in which they were more abundant in the obesity group, whereas *Streptococcus sanguinis* dominated in the normal weight group. People with obesity had a higher number of salivary microbial genes [6].

OBJECTIVES

The aim of the study was to discover the colonization resistance state of the oral mucosa of young patients with different body mass indices and their dependence on the intensity of the teeth carious and inflammatory response of the gums.

MATERIAL AND METHODS

This research was a part of a study devoted to the discovery of the origins and development of periodontal disease in young people with obesity. Clinical examination was conducted among 132 Ukrainians of all genders, with age ranging from 18 to 22 years.

The design of the study as well as the therapeutic and diagnostic measures agreed with commission on bioethics of the Poltava State Medical University (No. 197), and all participants signed a written consent. The inclusion criteria were young patients aged 18-22 years. The groups of obese patients had alimentary obesity, without any endocrine diseases diagnosed by a general doctor.

The exclusion criteria were endocrine diseases, pregnancy, breastfeeding, drug use, alcoholism, mental illness, participation in other clinical trials 1 month before the inclusion in this study, presence of active tuberculosis, viral hepatitis, and presence of fixed orthodontic appliance in the oral cavity.

In all patients, body weight, height, waist and hip ratio and body mass index were determined, which was as a criterion for division into groups. According to body mass index, four groups were created: the first group consisted of 33 patients with normal body mass index (BMI: 18.5-24.9 kg/m^2); the second group included 36 patients with excess weight (BMI: 25.0-29.9 kg/m^2), the third group – 31 patients with 1st degree obesity (BMI: 30.0-34.9 kg/m^2), the fourth group comprised of 32 patients with 2nd degree obesity (BMI: 35.0-39.9 kg/m^2).

Initial oral cavity status was detected by dental index assessment as an evaluation of caries intensity by decay, missing, and filled teeth (DMFT) [7]. Oral hygiene index (OHI) and papilla bleeding index (PBI) were determined in all groups [8, 9]. Periodontal chart was completed for all patients. Periodontal diagnosis was defined according to classification of periodontal and peri-implant diseases and conditions (November 9-11, 2017, Chicago) [10].

Screening of colonization resistance in the oral mucosa was determined by microscopy evaluation of the buccal epithelium, according to the method proposed by Petrushanko and Chereda [3]. This method entails the determination of the number of oral streptococci adhered to buccal epithelial cells in a smear stained by Romanowski-Gimza. Adhesive number (AN) was defined as the average number of streptococci adhered to

1 epitheliocyte. Adhesive index (AI) was the percentage of epitheliocytes that adhered to more than 10 streptococci. Qualitative assessment of colonization resistance was performed according to a scoring system. Colonization resistance index (CRI) 0 corresponded to AN < 20, AI < 50% indicated suppression of colonization resistance and reducing antagonistic properties of oral microflora (Figure 1). CRI 1 related to AN 20-60, AI > 50% indicated a high level of colonization resistance of the oral cavity. CRI 2 corresponded to AN > 60 and AI = 100%, which indicated an increased tension of the colonization barrier and an increase in the number of microorganisms that can be both opportunistic and pathogenic (Figure 2). Microscopy of buccal epithelial samples was performed using a light microscopy, with a magnification of ×400.

OriginPro 8.5.1.315 was applied for statistical processing. All results were described as average and standard errors. For data analysis, one-factor analysis of variance (one-way ANOVA) for unrelated samples and corrections Bonferroni for multiple comparisons were performed. Difference between groups was considered statistically significant at $p < 0.05$. Statistical processing of data in percentage was processed by a method of variation statistics according to Oyvin.

RESULTS

The average mean and standard error of BMI for the patients of the 1st group were 22.69 ± 0.29 kg/m^2, for the patients of the 2nd group were 27.84 ± 0.21 kg/m^2, in the 3rd group, they were 32.0 ± 0.28 kg/m^2, and 38.18 ± 0.68 kg/m^2 in the 4th group of patients.

The prevalence of caries in all examined groups was on average 97.7%, which corresponds to the results of several epidemiological researchers performed among the Ukrainian population. The intensity of carious process (DMFT) in the 1st group was 5.88 ± 0.67, in the 2nd group – 6.84 ± 0.58, in the 3rd group – 7.11 ± 1.07, and in the 4th group – 5.96 ± 0.84.

OHI in the 1st group was 1.17 ± 0.07, in the 2nd group – 0.95 ± 0.07, in the 3rd group – 1.42 ± 0.1, and in the 4th group – 1.4 ± 0.06. PBI was 2.09 ± 0.35 in the 1st group, 3.16 ± 0.37 in the 2nd, 4.26 ± 0.4 in the 3rd, and 3.9 ± 0.34 in the 4th groups.

45.5% patients of the 1st group had intact periodontium, 54.5% were diagnosed with biofilm-associated alone gingivitis. In the 2nd group, 25% had intact periodontium, and 75% had biofilm-associated alone gingivitis. In the 3rd group, 19.4% had intact periodontium, in 13% were diagnosed with biofilm-associated alone gingivitis and in 67.74% presented non-dental plaque-induced gingivitis. In the 4th group, 9.4% had intact periodontium and with biofilm-associated alone gingivitis and 81.2% were diagnosed with non-dental plaque-induced gingivitis.

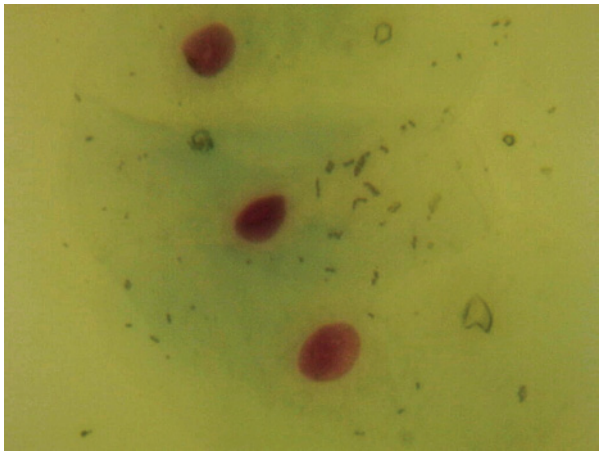

FIGURE 1. Photo of the sample of the buccal epithelium of patient K. with CR 0 (AI = 42; AN = 12.92), magnification ×400

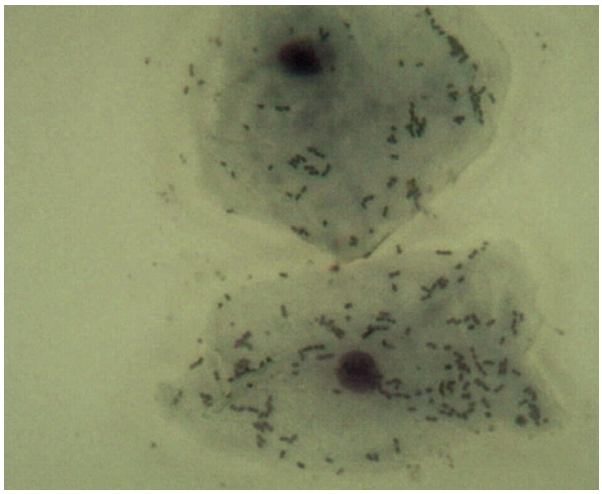

FIGURE 2. Photo of the sample of the buccal epithelium of patient D. with CR 2 (AI = 100; AN = 46.18), magnification ×400

In the 1st group, the average mean and standard error of AN were 24.06 ± 2.6 and AI = 62.5 ± 3.15. In the 2nd group, AN = 23.06 ± 3.0 and AI = 61.31 ± 5.35. In the 3rd group, AN = 19.6 ± 1.56 and AI = 60.51 ± 4.31. In the 4th group, AN = 19.24 ± 1.27 and AI = 64.68 ± 3.46.

This corresponded to the following values of colonization resistance index (CRI). In the 1st group, 21.2% of patients had a CRI 0, 78,8% – CRI 1. In the 2nd group, 44.4% of patients had a CRI 0, 38.9% – CRI 1, 16.7% had CRI 2. In the 3rd group, 64.5% had CRI 0, 25.8% – CRI 1, 9.7% had CRI 2. In the 4th group, 68.75% of patients had CRI – 0, 31.25% – CRI 1. The results on screening of colonization resistance of the oral mucosa of young patients, considering the intensity of carious process, are shown in Table 1. Screening of colonization resistance of the oral mucosa of young patients, considering the condition of gum health is shown in Table 2.

TABLE 1. State of colonization resistance of the oral cavity of young people with different BMI depending on intensity of the carious process

	Group I ($n = 33$)		Group II ($n = 36$)		Group III ($n = 31$)		Group IV ($n = 32$)	
	DMFT < 6, $n = 12$	DMFT > 6, $n = 21$	DMFT < 6, $n = 16$	DMFT > 6, $n = 20$	DMFT < 6, $n = 18$	DMFT > 6, $n = 13$	DMFT < 6, $n = 13$	DMFT > 6, $n = 19$
CR 0	8.3 ± 4.8%	42.9 ± 8.6%	6.2 ± 4%	5.0 ± 3.6%	55.5 ± 8.9%	69.0 ± 8.3%	75.9 ± 7.5%	31.0 ± 8.2%
CR 1	91.7 ± 4.8%	57.1 ± 8.6%	93.8 ± 4%	65.0 ± 7.9%	44.5 ± 8.9%	8.0 ± 4.8%	24.1 ± 7.5%	69.0 ± 8.2%
CR 2	0.0%	0.0%	0.0%	30.0 ± 7.6%	0.0%	23.0 ± 7.5%	0.0%	0.0%

TABLE 2. State of colonization resistance of the oral cavity of young patients with different BMI depending on inflammatory process in the gums

	Group I ($n = 33$)		Group II ($n = 36$)		Group III ($n = 31$)		Group IV ($n = 32$)	
	Intact periodontium $n = 15$	Gingivitis $n = 18$	Intact periodontium $n = 9$	Gingivitis $n = 27$	Intact periodontium $n = 6$	Gingivitis $n = 25$	Intact periodontium $n = 3$	Gingivitis $n = 29$
CR 0	13.34 ± 5.9%	44.5 ± 8.6%	22.2 ± 6.9%	51.85 ± 8.32%	0.0%	80.0 ± 7.2%	0.0%	82.7 ± 6.6%
CR 1	86.6 ± 5.9%	55.5 ± 8.6%	77.8 ± 6.9%	25.93% ± 7.3%	100.0%	8.0 ± 4.8%	100.0%	17.3 ± 6.6%
CR 2	0.0%	0.0%	0.0%	22.22 ± 6.9%	0.0%	12.0 ± 5.8%	0.0%	0.0%

DISCUSSION

The most important task of modern dentistry is not concentrating on the development of new treatment methods, but prevention, which is the only correct and reasonable way to maintain and improve the oral health. Prevention should be primarily targeted, aiming at diagnosing and elimination of certain patient-specific risk factors for the occurrence and progression of disease. These methods should be easy to use; thus, they can be applied in routine diagnostics, and should have sufficient diagnostic value.

There were no significant differences found between the carious process intensity and BMI. The severity of the carious process did not depend on BMI value. There was no correlation between individuals with DMFT < 6 and DMFT > 6 and BMI values (Table 1). The intensity of caries may depend on some other aspects, such as teeth mineralization and structural and functional resistance [11, 12].

The percentage of patients with periodontal disease was higher in patients with BMI > 30.00 kg/m^2. In the fourth group, the number of people with gingivitis was 90.6% and in the 3rd group, 80.6% of patients had gingivitis compared with patients of the 1st and 2nd groups, where the incidence was significantly lower, with 54.5% and 75.0%, respectively (Table 2). No significant differences were found between AN and AI in any of the examined group. There was a significant correlation between colonization resistance and intensity of the caries process. Therefore, in patients with high caries intensity DMFT > 6 in all groups, CRI 0 and CRI 2 prevailed, which indicate the tension of the colonization resistance barrier and the suppression of the colonization resistance barrier of oral mucosae. The percentage of patients with CRI 0 and CRI 2 also prevailed among those with 1st and 2nd degree obesity and high caries intensity. Hence, the number of people with DMFT > 6 exceeded predominantly CRI 0 or CRI 2, which indicated a higher tension of the microbial barrier compared with people with low caries intensity (DMFT < 6), where CRI 1 score indicated a high level of colonization resistance. In summary, high intensity of carious led to a disturbance of the colonization resistance barrier, especially in those with a BMI higher than 30 kg/m^2. 90.6% and 80.6% of individuals with 1st and 2nd degree obesity had periodontal disease compared with normal BMI and overweight patients, where the prevalence of gingivitis was significantly lower.

In patients with intact periodontium, high colonization resistance CRI, CRI 1 was predominant; in the first group, it was 86.66%, in the second, 77.8%, in the third, 100% and in the fourth, it was 100%. Higher intensity of colonization resistance was observed in patients with gingivitis, as was assessed by the predominance of CRI 0 and CRI 2. In obese individuals (groups 3rd and 4th) with gingivitis, number of individuals with impaired colonization resistance was much higher than in patients with normal BMI. Therefore, 92% of patients with 1st degree obesity, and 83% of patients with 2nd degree obesity and gingivitis had tense resistance of colonization barrier.

According to OHI values, the level of oral hygiene was assessed as satisfactory (range, 0.7-1.6), which was observed in all groups of patients. The values of PBI in-

dex were directly proportional to the intensity of inflammatory process and the severity of gum damage.

In our opinion, the primary factor in the development of inflammatory diseases of the periodontium that are not associated with dental plaque are systemic changes in the organism, which lead to disruption of oral cavity homeostasis, i.e., colonization resistance of oral mucosa. In the present study low level of colonization resistance (CRI 0 and CRI 2) was observed in 70% of obese patients compared with individuals with normal BMI. While, the low level of colonization resistance was observed only in 21% (CRI 0 and CRI 2), in overweight individuals, the number of people with impaired colonization resistance was 39%. The higher prevalence and intensity of gingivitis in patients with 1st and 2nd degree obesity can explain the origin of gingivitis, which is not associated with dental plaque in obese individuals. Although oral hygiene in all examined patients of all groups was in the range 0.9-1.6, and was considered satisfactory, the higher intensity and prevalence of gingivitis in obese individuals can be explained by the fact that an excess of subcutaneous and visceral fat triggered chronic mild systemic inflammation, oxidative stress alteration, and insulin resistance as well as vegetative disturbance, which plays a significant role in the pathogenesis of periodontal disease in the background of obesity [13].

Some studies have indicated a huge impact of obesity on oral health. Monosodium glutamate-induced obesity in rats triggered periodontal tissue alterations. The imbalance of protein-inhibitory capacity, low antioxidative activity, significantly higher content of fucosylated proteins and proteoglycans was observed in the gingival tissues of obese rats, compared with normal body mass index rats [14]. Obese individuals have significantly higher pro-oxidant activity and activation of oxidative and nitrosative stress, as demonstrated by an increased content of oxidatively modified proteins and phospholipids in the saliva [15]. These changes indicate that obesity are associated with a development of constant mild systemic inflammation, which maintains and leads to a more severe course of all inflammatory processes in the body. This is caused by the presence of excess visceral and subcutaneous fat. Adipocytes secrete into the bloodstream more pro-inflammatory adipocytokines, which create a systemic pro-inflammatory background [16]. Obese patients have a more severe gingivitis than patients with normal body weight, as evidenced by a higher prevalence of the disease, intensity of the inflammatory process and changes in biochemical parameters of the saliva [17].

CONCLUSIONS

Mild systemic inflammation in obese patients is a significant risk factor that alternate the local protective mechanisms of the oral mucosa. Patients with 1st and 2nd degree obesity have suppression of colonization resistance of the oral mucosa and higher intensity of gum alteration compared with individuals with normal BMI.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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