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THE QUALITATIVE AND QUANTITATIVE STRUCTURE OF ORAL MICROBIOCENOSIS IN RATS WITH PERIODONTITIS IN A SETTING OF HYPER- AND HYPOTHYROIDISM

Vitaliy Shcherba [1], ***Inna Krynytska*** [2], ***Mariya Marushchak*** [2,✉], ***Mykhaylo Korda*** [3]

[1] Department of Dentistry Horbachevsky Ternopil National Medical University, Ternopil, Ukraine
[2] Department of Functional and Laboratory Diagnostics Horbachevsky Ternopil National Medical University, Ternopil, Ukraine
[3] Department of Medical Biochemistry Horbachevsky Ternopil National Medical University, Ternopil, Ukraine

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Abstract

Background and aims. *Oral microbial flora is a highly sensitive indicator system producing qualitative and quantitative responses to changes in various individual organs and systems. The aim of study was to perform a comparative analysis of qualitative and quantitative structure of oral microbiocenosis in rats with comorbidity-free periodontitis and in animals with periodontitis in a setting of hyper- and hypothyroidism.* ***Material and methods.*** *Experimental studies were conducted on 48 mature male white rats. Samples for microbiological tests were taken from dental surfaces (on the border between hard tissue and gums in the interdental spaces). The isolated pure cultures were identified by their morphological, tinctorial, cultural and biochemical properties and the signs of pathogenecity.* ***Results.*** *We found that the oral dysbiosis occurring in a setting of periodontitis in rats is chiefly characterized by increased quantity of coccal forms and by increased candidal inoculation; these organisms cumulatively inhibit the growth of normal microbial flora, such as Lactobacilli, bacteroids and Bifidobacteria. Thyroid dysfunction exacerbates changes in the qualitative and quantitative structure of oral microbiocenosis.* ***Conclusions.*** *The periodontitis in a setting of thyroid dysfunction increases both the species variety and the quantitative counts of oral microbial flora, with predominance of such microbial organisms as Staph. aureus, E. coli, E. faecalis, Candida albicans and P. aeruginosa.*

key words: *thyroid alterations, periodontitis, microbiocenosis.*

Background and aims

The prevalence of thyroid disorders in adult population from normal iodine intake regions was found to vary between 6.6 and 8.8% [1]. Thyroid disorders have registered an increasing incidence since the Chernobyl nuclear fallout in 1986, mainly in the Central, Northern and Eastern Europe. In Ukraine, over the last 5 years, the number of thyroid disorders has increased by 5 times and their proportion in the structure of endocrine diseases is 47.3 % [2,3]. Thyroid

✉ Majdan Voli 1, Ternopil 46001, Ukraine.
corresponding author e-mail: marushchak@tdmu.edu.ua

hormones regulate numerous metabolic processes. Therefore, any alteration in their synthesis or function has important health implications. However, limited data are available regarding the relationship between thyroid hormone imbalance and periodontal health [4,5]. Periodontitis is an immune inflammatory response which arises from the interaction between the periodontal-pathogenic bacteria and the host, thus, dysbiosis play an important role in its pathogenesis [6,7]. In periodontitis, the severity of dysbiosis directly correlates with poor oral hygiene, higher periodontal indices and the severity of tooth decay [8]. The pathophysiological basis for abnormal composition of microbial flora mainly includes immune dysfunction, with phagocytic functional disorganization being the primary factor. A special place among the factors of phagocyte-mediated immunity belongs to antimicrobial peptides. On the one hand, these peptides are natural endogenous antibiotics and on the other hand they act as signal molecules involved in activation of immune cells and tissue repair [9].

Oral microbial flora is a highly sensitive indicator system producing qualitative and quantitative responses to changes in various individual organs and systems and in the body as a whole [10]. Despite the plethora of current information on the role of qualitative and quantitative changes of microbial flora in patients with chronic generalized periodontitis [11-14], no data on such changes in patients with thyroid dysfunction could be found in the available literature.

Therefore, the objective of our study was to perform a comparative analysis of qualitative and quantitative structure of oral microbiocenosis in rats with comorbidity-free periodontitis and in animals with periodontitis in a setting of hyper- and hypothyroidism.

Material and methods

The microbiological tests were performed in 48 inbred mature male white rats with a body weight of 180-200 g kept on a standard vivarium diet.

Experimental animals were divided into the following groups: Group I: control animals administered with intragastric 1% starch solution (n=12); Group II: animals with a model of periodontitis. For 2 weeks, the rats in this group were administered with 40 µL (1 mg/mL) of E. coli lipopolysaccharide (LPS) (manufactured by Sigma-Aldrich, USA) into gingival tissues every other day (n=12) [15]; Group III: rats with periodontitis in a setting of hyperthyroidism. To create an experimental model of thyroid hyperfunction, the animals (n=12) received daily intragastric doses of L-thyroxine in 1% starch solution at 10 µg/day per 100 g of body weight for 21 days [16]. Starting with Day 8 of the experiment, the rats were given LPS into gingival tissue for 2 weeks; Group IV included rats with periodontitis in a setting of hypothyroidism. To create an experimental model of thyroid hypofunction [16], the animals (n=12) received daily intragastric doses of methimazole in 1% starch solution at 1 mg/day per 100 g of body weight for 21 days. Starting from Day 8 of the experiment, the rats were given LPS into gingival tissue for 2 weeks. Rats were sacrificed by exsanguination under thiopental-sodium anesthesia on Day 22 from the onset of the experiment.

All manipulations with experimental animals were performed according to provisions of the *European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes* [17].

To confirm hyper- and hypothyroid status, serum levels of free thyroxine (FT4), free triiodothyronine (FT3) and thyroid stimulating

hormone (TSH) were assayed with ELISA using the kits manufactured by Vector Best (Russia).

Microbiological tests were based at the Department of Microbiology, Virology and Immunology of I. Horbachevsky Ternopil National Medical University. Samples were taken from dental surfaces (on the border between hard tissue and gums in the interdental spaces) using a standard sterile swab of a Sarstedt transport system (Germany).

Staphylococci were isolated by inoculating *BD Baird-Parker Agar* culture medium with washes. Enterococci were isolated by inoculating *Bile Esculin Azide Agar* (manufactured by HiMedia); Streptococci were isolated by inoculating *Streptococcus Selective Agar* (manufactured by HiMedia). Enterobacteriaceae (Escherichiae, Proteus, Klebsiella, etc.) were grown on Endo agar, Eosin methylene blue (Levine's formulation) and on Ploskirev baktoagar manufactured by Farmactiv (Kyiv, Ukraine). Isolation of *Pseudomonas aeruginosa* was carried out on *Pseudomonas Isolation Agar* (manufactured by HiMedia); bacteria of *Lactobacillus genus* were isolated on Lactobacagar manufactured by Farmactiv (Kyiv, Ukraine). The cultures were incubated in a thermostat at 37±1°C for 24 to 48 hours. Cultures of *Bifidobacteria* were obtained on Bifidum growth medium and cultures of bacteroids were obtained on Schaedler agar +5 % sheep erythrocytes under anaerobic conditions in a microanaerostat manufactured by Bio Merieux at 37±1°C for 24–48 hours. Fungi were grown on a Sabouraud agar with chloramphenicol 400 mg/dm^3 at 28±1°C for 3 to 5 days.

The isolated pure cultures were identified by their morphological, tinctorial, cultural and biochemical properties and the signs of pathogenecity [18]. To this end, the authors were using such diagnostic test systems as "STAPHY-test 16", "STREPTO-test 16", EN-COCCUS-test and "ENTERO-test 24" (manufactured by LACHEMA, Czech Republic) and "API20CAUX" API Candida (manufactured by bioMerieux, France). Lactobacteriaceae were assessed using a PBDL plate (Nizhny Novgorod, Russia). The results of quantitative testing informed microbial flora content, which was expressed in colony-forming units (CFUs) per 1 mL of the wash.

Statistical analysis of study results was performed using generally accepted variation statistics methods with STATISTICA 7.0 software package. Quantitative characteristics of the parameters were given as median and quartiles (lower and upper quartile), i.e. Me (Lq; Uq). Given the non-normal distribution of values, the comparative analysis across the four groups was performed using nonparametric Kruskall-Wallis test. When significant values were obtained with the latter test ($p<0.05$), further pair-wise comparison between the groups was performed using Mann–Whitney U test while taking into consideration the Bonferroni correction for assessment of p-values.

Results

In rats with a model of periodontitis, the number of *Staphylococcus spp.* strains was increased 19.9-fold ($p<0.001$) relative to the control group (see Table 1). With periodontitis in a setting of hyperthyroidism, the intensity of colonization with *Staphylococcus spp.* was increased by 26.4 times vs control group ($p<0.001$); in rats with periodontitis in a setting of hypothyroidism the respective superiority was 27.5 times ($p<0.001$). When comparing the counts of *Staphylococcus spp.* in rats with periodontitis and thyroid dysfunction to the animals with comorbidity-free periodontitis, significantly higher counts were found in both hyperthyroid rats (by 32.9%) and hypothyroid

rats (by 38.1%). In the meantime, no significant differences were found between the animals with periodontitis in a setting of hyper- and hypothyroidism.

Table 1. The intensity of oral colonization in rats with periodontitis without other comorbidities and in a setting of hyper- and hypothyroidism (Me (Lq; Uq)

Microbial species	Group of animals			
	Control (Group 1)	Periodontitis (Group 2)	Periodontitis in a setting of hyperthyroidism (Group 3)	Periodontitis in a setting of hypothyroidism (Group 4)
Microbial counts in the wash, CFU/cm^3				
Staphylococcus spp	3.35×10^1 (2.85×10^1; 3.50×10^1)	6.66×10^2 (5.95×10^2; 6.98×10^2)	8.85×10^2 (8.10×10^2; 9.60×10^2)	9.20×10^2 (8.70×10^2; 9.70×10^2)
	Kruskall-Wallis test, H=40.05, p<0.001			
	p_{1-2}<0.001* p_{1-3}<0.001* p_{1-4}<0.001*	p_{2-3}<0.001* p_{2-4}<0.001*	p_{3-4}=0.273	–
Staph. aureus	0 (0; 0)	2.42×10^2 (0; 2.55×10^2)	8.45×10^2 (7.70×10^2; 9.30×10^2)	5.20×10^2 (4.80×10^2; 5.50×10^2)
	Kruskall-Wallis test, H=40.86, p<0.001			
	p_{1-2}=0.006* p_{1-3}<0.001* p_{1-4}<0.001*	p_{2-3}<0.001* p_{2-4}<0.001*	p_{3-4}<0.001*	–
Streptococcus spp.	1.91×10^4 (1.85×10^4; 1.94×10^4)	3.80×10^4 (3.35×10^4; 4.45×10^4)	7.40×10^4 (6.75×10^4; 8.00×10^4)	4.38×10^4 (3.93×10^4; 4.86×10^4)
	Kruskall-Wallis test, H=40.56, p<0.001			
	p_{1-2}<0.001* p_{1-3}<0.001* p_{1-4}<0.001*	p_{2-3}<0.001* p_{2-4}=0.069	p_{3-4}<0.001*	–
Enterobacteriaceae	1.11×10^2 (0.90×10^2; 3.50×10^2)	3.55×10^2 (3.10×10^2; 3.80×10^2)	5.35×10^2 (5.00×10^2; 5.55×10^2)	4.95×10^2 (4.65×10^2; 5.25×10^2)
Kruskall-Wallis test, H=40.91, p<0.001				
	p_{1-2}<0.001* p_{1-3}<0.001* p_{1-4}<0.001*	p_{2-3}<0.001* p_{2-4}<0.001*	p_{3-4}=0.033	–
E. coli	0	0 (0; 0.7×10^2)	3.52×10^2 (0; 3.65×10^2)	1.4×10^2 (0; 3.15×10^2)
Kruskall-Wallis test, H=16.05, p=0.001				
	p_{1-2}=0.033 p_{1-3}=0.006* p_{1-4}=0.038	p_{2-3}=0.021 p_{2-4}=0.167	p_{3-4}=0.061	–
Enterococcus spp.	4.15×10^2 (3.80×10^2; 4.78×10^2)	5.65×10^2 (5.20×10^2; 5.85×10^2)	8.15×10^2 (7.70×10^2; 8.60×10^2)	7.95×10^2 (7.40×10^2; 8.35×10^2)
Kruskall-Wallis test, H=38.06, p<0.001				
	p_{1-2}<0.001* p_{1-3}<0.001* p_{1-4}<0.001*	p_{2-3}<0.001* p_{2-4}<0.001*	p_{3-4}=0.436	–
E. faecalis	0	0.85×10^2 (0; 1.05×10^2)	7.23×10^2 (6.45×10^2; 7.95×10^2)	6.55×10^2 (0; 7.00×10^2)
Kruskall-Wallis test, H=19.06, p<0.001				
	p_{1-2}=0.046 p_{1-3}<0.001* p_{1-4}=0.015	p_{2-3}=0.002* p_{2-4}=0.050	p_{3-4}=0.133	–

Table 1. *Continued.*

Microbial species	Group of animals			
	Control (Group 1)	Periodontitis (Group 2)	Periodontitis in a setting of hyperthyroidism (Group 3)	Periodontitis in a setting of hypothyroidism (Group 4)
Yeast-like fungi	0.26×10^3 (0.21×10^3; 0.29×10^3)	4.65×10^3 (4.20×10^3; 4.90×10^3)	7.45×10^3 (6.90×10^3; 7.85×10^3)	6.15×10^3 (5.70×10^3; 6.60×10^3)
Kruskall-Wallis test, H=41.87, p<0.001				
	p_{1-2}<0.001* p_{1-3}<0.001* p_{1-4}<0.001*	p_{2-3}<0.001* p_{2-4}<0.001*	p_{3-4}=<0.001*	–
Candida albicans	0	0 (0; 0.61×10^3)	2.90×10^3 (2.70×10^3; 3.05×10^3)	0.80×10^3 (0.74×10^3; 0.84×10^3)
Kruskall-Wallis test, H=42.28, p<0.001				
	p_{1-2}=0.167 p_{1-3}<0.001* p_{1-4}<0.001*	p_{2-3}<0.001* p_{2-4}<0.001*	p_{3-4}<0.001*	–
Lactobacillus spp.	7.35×10^4 (6.98×10^4; 7.65×10^4)	0.83×10^4 (0.76×10^4; 0.85×10^4)	0.24×10^4 (0.22×10^4; 0.27×10^4)	0.21×10^4 (0.19×10^4; 0.24×10^4)
Kruskall-Wallis test, H=40.58, p<0.001				
	p_{1-2}<0.001* p_{1-3}<0.001* p_{1-4}<0.001*	p_{2-3}<0.001* p_{2-4}<0.001*	p_{3-4}=0.065	–
Bifidobacterium spp.	8.55×10^4 (8.30×10^4; 9.55×10^4)	0.54×10^4 (0.50×10^4; 0.58×10^4)	0.27×10^4 (0.25×10^4; 0.31×10^4)	0.24×10^4 (0.24×10^4; 0.27×10^4)
Kruskall-Wallis test, H=40.31, p<0.001				
	p_{1-2}<0.001* p_{1-3}<0.001* p_{1-4}<0.001*	p_{2-3}<0.001* p_{2-4}<0.001*	p_{3-4}=0.133	–
Bacteroids	9.55×10^4 (9.30×10^4; 10.55×10^4)	0.50×10^4 (0.45×10^4; 0.54×10^4)	0.17×10^4 (0.12×10^4; 0.20×10^4)	0.13×10^4 (0.10×10^4; 0.17×10^4)
Kruskall-Wallis test, H=21.03, p<0.001				
	p_{1-2}<0.001* p_{1-3}<0.001* p_{1-4}<0.001*	p_{2-3}<0.001* p_{2-4}<0.001*	p_{3-4}=0.112	–
P. aeruginosa	0	0 (0; 0.39×10^2)	2.00×10^2 (0.9×10^2; 2.45×10^2)	0.74×10^2 (0; 1.11×10^2)
Kruskall-Wallis test, H=16.05, p=0.001				
	p_{1-2}=0.166 p_{1-3}=0.002* p_{1-4}=0.015	p_{2-3}=0.006* p_{2-4}=0.079	p_{3-4}=0.012*	–
Total microbial count, CFU/mL of the wash	0.99×10^5 (0.93×10^5; 1.01×10^5)	1.74×10^5 (1.48×10^5; 1.97×10^5)	3.50×10^5 (3.14×10^5; 3.95×10^5)	3.25×10^5 (3.01×10^5; 3.54×10^5)
Kruskall-Wallis test, H=40.21, p<0.001				
	p_{1-2}<0.001* p_{1-3}<0.001* p_{1-4}<0.001*	p_{2-3}<0.001* p_{2-4}<0.001*	p_{3-4}=0.157	–

Note 1. The level of statistical significance according to Bonferroni adjustment for intergroup comparison is at p<0.008.
Note 2. p_{1-2} = the probability of Mann–Whitney U test when comparing Group 1 and Group 2.
Note 3. p_{1-3} = the probability of Mann–Whitney U test when comparing Group 1 and Group 3.

Note 4. p_{1-4} = the probability of Mann–Whitney U test when comparing Group 1 and Group 4.
Note 5. p_{2-3} = the probability of Mann–Whitney U test when comparing Group 2 and Group 3.
Note 6. p_{2-4} = the probability of Mann–Whitney U test when comparing Group 2 and Group 4.
Note 7. p_{3-4} = the probability of Mann–Whitney U test when comparing Group 3 and Group 4.
Note 8. * = statistically significant results.

S. aureus was not isolated from control animals in our study; however it was found in 8 rats with a model of periodontitis (at 2.42×10^2 CFU/cm^3). When comparing the counts of *S. aureus* in rats with periodontitis and thyroid dysfunction to the animals with comorbidity-free periodontitis, significantly higher counts were found in both hyperthyroid rats (a 3.5-fold difference, $p<0.001$) and in hypothyroid rats (a 2.1-fold difference, $p<0.001$). That said, the intensity of colonization with *S. aureus* in hyperthyroid rats was 1.6 times ($p<0.001$) higher than that in hypothyroid animals.

The rats with a model of periodontitis had a 2.0 times higher counts of *Streptococcus spp.* ($p<0.001$) than rats in the control group. With periodontitis in a setting of hyperthyroidism, the intensity of colonization with *Streptococcus spp.* was increased 3.9 times relative to the control group ($p<0.001$); in rats with periodontitis in a setting of hypothyroidism the respective superiority was 2.3-fold ($p<0.001$).

When comparing the counts of *Streptococcus spp.* in rats with periodontitis and thyroid dysfunction to the animals with comorbidity-free periodontitis, significantly higher counts were found only in hyperthyroid rats (a 1.9-fold difference). That said, the intensity of colonization with *Streptococcus spp.* in hyperthyroid rats was 1.7 times ($p<0.001$) higher than that in hypothyroid animals.

The rats with a model of periodontitis had a 3.2 times higher count of *Enterobacteriaceae* ($p<0.001$) than rats in the control group. With periodontitis in a setting of hyperthyroidism, the intensity of colonization with *Enterobacteriaceae* was increased 4.8 times relative to the control group ($p<0.001$); in rats with periodontitis in a setting of hypothyroidism the respective superiority was 4.5-fold ($p<0.001$). When comparing the counts of *Enterobacteriaceae* in rats with periodontitis and thyroid dysfunction to the animals with comorbidity-free periodontitis, significantly higher counts were found in both hyperthyroid rats (by 50.7%) and hypothyroid rats (by 39.4%). In the meantime, no significant differences were found between the animals with periodontitis in a setting of hyper- and hypothyroidism.

In our study, *E. coli* was not isolated from control animals; it was, however, isolated from 4 rats with a model of periodontitis 0 (0; 0.7×10^2) CFU/cm^3. When comparing the counts of *E. coli* in rats with periodontitis and thyroid dysfunction to the animals with comorbidity-free periodontitis, significantly higher counts were found in both hyperthyroid and hypothyroid rats. In the meantime, no significant differences were found between the animals with periodontitis in a setting of hyper- and hypothyroidism.

In rats with a model of periodontitis, the intensity of colonization with strains of *Enterococcus spp.* was increased 36.1% ($p<0.001$) relative to the control group. With periodontitis in a setting of hyperthyroidism, this parameter was increased 2.0 times relative to the control group ($p<0.001$), while in rats with periodontitis in a setting of hypothyroidism the respective superiority was 1.9-fold ($p<0.001$). When comparing the counts of *Enterococcus spp.* in rats with periodontitis and thyroid dysfunction to the animals with comorbidity-free periodontitis, significantly higher counts were found in both hyperthyroid rats (by 44.2%) and in hypothyroid rats (by 40.7%). In the meantime, no significant differences were found between

the animals with periodontitis in a setting of hyper- and hypothyroidism.

E. faecalis was detected in 7 rats with a model of periodontitis in our study (at 0.85×10^2 CFU/cm^3). When comparing the counts of *E. faecalis* strains in rats with periodontitis and thyroid dysfunction to the animals with comorbidity-free periodontitis, significantly higher counts were found in hyperthyroid rats (an 8.5-fold difference, p=0.002). The respective changes in hypothyroid rats were not found to be significant.

Of note, there were large quantities of yeast-like fungi in rats with a model of periodontitis, a 17.9-fold superiority (p<0.001) relative to the control group. With periodontitis in a setting of hyperthyroidism, the intensity of colonization with yeast-like fungi was increased 28.6 times relative to the control group (p<0.001); in rats with periodontitis in a setting of hypothyroidism, the respective superiority was 23.6-fold (p<0.001). When comparing the counts of *Candida* in rats with periodontitis and thyroid dysfunction to the animals with comorbidity-free periodontitis, significantly higher counts were found in both hyperthyroid rats (a 1.6-fold difference) and in hypothyroid rats (a 1.3-fold difference). That said, the intensity of colonization with *Candida* strains in hyperthyroid rats was 21.1% (p<0.001) higher than that in hypothyroid animals.

In our study, *Candida albicans* was not isolated from control animals; it was, however, isolated from 4 rats with a model of periodontitis 0 (0; 0.61×10^3) CFU/cm^3. That said, the intensity of colonization with *Candida albicans* strains in rats with periodontitis in a setting of hyperthyroidism was 3.6 times (p<0.001) higher than that in hypothyroid animals.

P. aeruginosa was not isolated from control animals; it was, however, isolated from 4 rats with a model of periodontitis 0 (0; 0.39×10^2) CFU/cm^3. That said, the intensity of colonization with *P. aeruginosa* strains in rats with periodontitis in a setting of hyperthyroidism was 2.7 times higher than that in hypothyroid animals (a significant difference).

The counts of *Lactobacillus spp* in rats with a model of periodontitis were 8.9 times lower (p<0.001) and the counts of *Bifidobacterium spp.* were 15.8 times lower relative to the control group. That said, the counts of bacteroids were significantly (19.1 times) reduced. This probably suggests a pronounced reduction in normal microbiota of oral cavity in a setting of periodontitis.

With periodontitis in a setting of hyperthyroidism, the intensity of colonization with *Lactobacillus spp.* was 30.6 times lower relative to the control group (p<0.001); in rats with periodontitis in a setting of hypothyroidism, the respective inferiority was 35.0-fold (p<0.001). When comparing the counts of *Lactobacillus spp.* in rats with periodontitis and thyroid dysfunction to the animals with comorbidity-free periodontitis, significantly reduced counts were found in both hyperthyroid rats (a 3.5-fold difference) and in hypothyroid rats (a 4.0-fold difference). In the meantime, no significant differences were found between the animals with periodontitis in a setting of hyper- and hypothyroidism.

As for *Bifidobacterium spp.,* with periodontitis in a setting of hyperthyroidism, the intensity of colonization with respective strains was 31.7 times lower relative to the control group (p<0.001); in rats with periodontitis in a setting of hypothyroidism, the respective inferiority was 35.6-fold (p<0.001). When comparing the counts of *Bifidobacterium spp.* in rats with periodontitis and thyroid dysfunction to the animals with comorbidity-free periodontitis, significantly reduced counts were found in both hyperthyroid rats (a 2.0-fold difference) and in

hypothyroid rats (a 2.3-fold difference). In the meantime, no significant differences were found between the animals with periodontitis in a setting of hyper- and hypothyroidism.

A similar trend was observed concerning bacteroids. With periodontitis in a setting of hyperthyroidism, the intensity of colonization with bacteroids was 56.2 times lower relative to the control group ($p<0.001$); in rats with periodontitis in a setting of hypothyroidism, the respective inferiority was 73.5-fold ($p<0.001$). When comparing the counts of bacteroids in rats with periodontitis and thyroid dysfunction to the animals with comorbidity-free periodontitis, significantly reduced counts were found in both hyperthyroid rats (a 2.9-fold difference) and in hypothyroid rats (a 3.8-fold difference). In the meantime, no significant differences were found between the animals with periodontitis in a setting of hyper- and hypothyroidism.

Total microbial count in rats with a model of periodontitis was increased 1.8 times ($p<0.001$) relative to the control group. With periodontitis in a setting of hyperthyroidism, this parameter was increased 3.5 times relative to the control group ($p<0.001$), while in rats with periodontitis in a setting of hypothyroidism, the respective superiority was 3.3-fold ($p<0.001$). When comparing total microbial counts in rats with periodontitis and thyroid dysfunction to the animals with comorbidity-free periodontitis, significantly higher counts were found in both hyperthyroid rats (a 2.0-fold difference) and in hypothyroid rats (a 1.9-fold difference). In the meantime, no significant differences were found between the animals with periodontitis in a setting of hyper- and hypothyroidism.

Discussion

Oral cavity is an ecological system populated with over 700 microbial species organized as a biofilm. One milliliter of saliva contains more than 10^8 microbial organisms of various species; buccal mucosal scraping may contain up to 10^{12} cells per 1 gram [19]. Oral cavity has favorable conditions for microbial growth. In part, these may include weakly alkaline *p*H, food residues and optimum humidity and temperature. Microbial organisms secrete biologically active substances, such as toxins and enzymes, which have strong toxic, allergenic and necrotic properties, leading to inflammation and tissue destruction. Periodontal pockets contain large amounts of white blood cells with a tendency to increase at various stages of periodontal disease. The obligate anaerobes of gingival pockets and periodontal pockets are believed to impact periodontal tissue directly with further progression of the disease [20].

The representatives of the so called “red complex” (*Porphyromonas gingivalis*, *Bacteroides forsytus* or *Tannerellaforsythia* and *Treponema denticola*) are reported in literature to possess the highest degree of pathogenecity against periodontal tissue [21]. There have been recent literature reports of herpes simplex virus, Epstein-Barr virus and cytomegalovirus detected in periodontal pockets, gingival biopsies and root canals in a setting of marginal and apical periodontal disease. Along with herpes viruses, representatives of periodontopathic microbial flora can be found in periodontal pockets. Therefore, the authors are making an assumption that viruses are playing a certain role in reproduction of aggressive microbial flora [22].

As reported by Hajishengallis G., the induction of periodontitis is a complex phenomenon, which involves the entire microbial population present in oral cavity [23]. Using the methods of molecular biology, Slocum C. et al. have demonstrated that over 300 bacterial species may colonize subgingival environment. Many of these species have been

suggested as potential periodontal pathogens [21]. There is a possibility that periodontal inflammation is to a great degree mediated by the so called "pathobionts". The latter are commensal organisms, which, in impaired homeostasis, have a potential to deregulate inflammatory responses and to cause disease [23-24]; alternative culprits may include "transbionts", i.e. the bacteria typical for other biotopes [22].

In rats with a model of periodontitis in our study, the number of *Staphylococcus spp.* strains very much increased vs the control group. Staphylococci are a large heterogeneous group of Gram-positive microbial organisms, which are divided into coagulase-positive and coagulase-negative organisms. They are capable of both forming their own biofilms and of acting as initiators of a biofilm process executed by other microbial organisms. Moreover, colonization of dental materials by *Staphylococci* and the subsequent biofilm formation is proven to destroy these materials [25]. When comparing the counts of *Staphylococcus spp.* in rats with periodontitis and thyroid dysfunction to the animals with comorbidity-free periodontitis, significantly higher counts were found in both hyperthyroid and hypothyroid rats.

Among coagulase-positive agents, *S. aureus* is the most well-known organism [26]. *S. aureus* was not isolated from control animals in our study; however it was found in 8 rats with a model of periodontitis. Heller D. et al. also observed the strains of *S. aureus* as one of the dominant species in patients with chronic periodontitis [27]. At the same time, Colombo A.P.V. et al. have found large quantities of *S. aureus* in subjects with a healthy periodontium compared to patients with aggressive periodontitis; however, there was no statistically significant difference compared to subjects with chronic periodontitis [28]. The intensity of colonization with *S. aureus* in hyperthyroid rats in our study was significantly higher than that in hypothyroid animals.

There is evidence that the streptococci present as part of microbiocenosis of dental plaque may cause inflammatory periodontal disease and tooth decay [29]. The rats with a model of periodontitis had higher counts of *Streptococcus spp.* vs control group. The intensity of colonization with *Streptococcus spp.* was significantly higher in rats with periodontitis and thyroid dysfunction vs rats with a model of periodontitis.

In our study, *E. coli* was not isolated from control animals; it was, however, isolated from 4 rats with a model of periodontitis. Our results are consistent with the findings of other authors, who point out the role of anaerobic Gram-negative *E. coli* in the pathogenesis of chronic periodontitis [30,31]. In a presence of thyroid dysfunction, the incidence and the intensity of colonization with strains of *E. coli* was increased in both hyperthyroid and hypothyroid rats. In the meantime, no significant differences were found between the animals with periodontitis in a setting of hyper- and hypothyroidism.

Enterococci are one of the most common causative agents of mixed anaerobic/aerobic infections. Being facultative aerobes, they are able to effectively stimulate the increase in quantity of obligate anaerobic pathogens by increasing their virulence potential [32]. *E. faecalis* was detected in 7 rats with a model of periodontitis in our study. When comparing the counts of *E. faecalis* strains in rats with periodontitis and thyroid dysfunction to the animals with comorbidity-free periodontitis, significantly higher counts were found in hyperthyroid rats.

There is evidence that *E. faecalis* may be regarded as a representative of periodontopathic microbial flora. Souto R. and Colombo A.P.

studied the incidence of *E. faecalis* colonization in sublingual biofilm and in saliva samples from people with chronic periodontitis and from healthy subjects. They have established positive correlations between the presence of *E. faecalis* and the clinical symptoms of periodontitis and biofilm accumulation [33]. Murad C.F. et al. also found *E. faecalis* and *S. warneri* to be part of the most prevalent bacterial species of the root canal microbiota associated with failure of endodontic treatment and that *E. faecalis* could play an important role in chronic periapical lesions [34]. Fritoli A. et al. also observed that in individuals with periodontitis *E. faecalis* and *Staph. warneri* were present at significantly higher mean levels than in the healthy individuals. Although the levels of *Staph. aureus* were also lower in healthy individuals, this difference between the two groups was not statistically significant [35].

Of note, there were large quantities of yeast-like fungi in rats with a model of periodontitis vs control group. When comparing the counts of *Candida* in rats with periodontitis and thyroid dysfunction to the animals with comorbidity-free periodontitis, significantly higher counts were found in both hyperthyroid and hypothyroid rats. High *Candida* detection rates may suggest reduction in nonspecific resistance and phagocytic immune responses as well as a reduced synthesis of secretory IgA [36].

In our study, *Candida albicans* was not isolated from control animals; it was, however, isolated from 4 rats with a model of periodontitis. There is evidence that *C. albicans* strains can be isolated not only from oral mucosa, but also from other oral regions, such as pulp cavity, carious lesions and periodontal pockets [37] Urzua B. et al. observed increased colonization with *Candida* strains in subgingival areas both in patients with chronic periodontitis and in patients with aggressive periodontitis compared to subjects with healthy periodontium [38]. The intensity of colonization with *Candida albicans* strains in rats with periodontitis in a setting of hyperthyroidism was significantly higher than that in hypothyroid animals.

P. aeruginosa was not isolated from control animals; it was, however, isolated from 4 rats with a model of periodontitis. That said, the intensity of colonization with *P. aeruginosa* strains in rats with periodontitis in a setting of hyperthyroidism was significantly higher than that in hypothyroid animals. As reported by O.V. Krysenko, *P. aeruginosa* strains are a frequent cause of pyoinflammatory periodontal disease and exacerbations of generalized periodontitis [36].

Therefore, when comparing the species composition of individual microbial organisms in rats of the control group and in the animals with a model of periodontitis, qualitative changes were found in composition of microbial flora, as manifest by such emerging strains as *Staph. aureus, E. coli*, *P. aeruginosa* and yeast-like fungi of the *Candida albicans* genus. When comparing the intensity of microbial colonization in rats with a model of periodontitis (both without concurrent disease and in a setting of thyroid dysfunction), the highest colonization rates have been documented for the strains of *Staph. aureus* and yeast-like fungi of the *Candida albicans* genus.

Conclusions

The oral dysbiosis occurring in a setting of periodontitis in rats is chiefly characterized by increased quantity of coccal forms and by increased candidal inoculation; these organisms cumulatively inhibit the growth of normal microbial flora, such as *Lactobacilli*, bacteroids and *Bifidobacteria*. The periodontitis in a setting of thyroid dysfunction increases both the species variety and the quantitative counts of oral microbial flora, with predominance of such

microbial organisms as *Staph. aureus, E. coli, E. faecalis, Candida albicans* and *P. aeruginosa*. Comparative assessment of intensity of oral microbial colonization in hyper- and hypothyroid animals with periodontitis has demonstrated significant changes only for the strains of *S. aureus* and *Candida*, which were predominant in hyperthyroid rats.

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