Does thyroid dysfunction influence inflammatory mediators in experimental periodontitis?

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Objective. The aim of the present study was to investigate the presence of inflammatory mediators in rats with only periodontitis and periodontitis in a setting of hyper- and hypo-thyroidism and to analyze the correlative linkages between inflammatory mediators and thyroid hormones.

Methods. White male 12–14 weeks old inbred rats ($n=48$) weighing 180–200 g were employed in the experiment. They were randomly divided into the following groups: Group I – control group, Group II – group with a model of periodontitis, Group III – group with a periodontitis in a setting of hyperthyroidism, and Group IV – group with periodontitis in a setting of hypothyroidism. The presence of tumor-necrosis factor- α (TNF- α) and interleukins IL-1 β and IL-10 in the periodontal homogenate supernatant was studied by a solid-phase enzyme-linked immunosorbent assay.

Results. It was shown that experimental lipopolysaccharide (LPS)-induced periodontitis is accompanied by hyperproduction of pro-inflammatory cytokines (TNF- α , IL-1 β) and reduction of anti-inflammatory cytokines (IL-10), whereas TNF- α underwent to maximum changes. Thyroid dysfunction exacerbates cytokine imbalance and severity of inflammation in experimental LPS-induced periodontitis, especially pronounced at hyperthyroidism, as evidenced by the predominance of TNF- α and IL-1 β levels in the periodontal homogenate supernatant by 38.5% ($p<0.01$) and 75.6% ($p<0.001$), respectively, hyperthyroid over the euthyroid, and by 20.1% ($p<0.05$) and 24.1% ($p<0.05$), respectively, over the hypothyroid rats.

Conclusions. Thyroid dysfunction, especially hyperthyroidism, may play an important role in the pro-inflammatory response in periodontitis. Hyperproduction of inflammatory mediators in thyroid dysfunction can induce a noticeable damage in the whole apparatus of the periodontium, thereby causing progression of periodontitis.

Key words: periodontitis, inflammation, hyperthyroidism, hypothyroidism, rats

Severe periodontitis, cavitated caries lesions and the consequence of tooth loss have become a disease burden, which is estimated at 12,900,000 disability-adjusted life years in 2015 or about 2% of the total human disease burden (Tonetti et al. 2017a, b). The Global Burden of Disease Study indicates that severe periodontitis is the 6th of most common diseases in the world, with an overall prevalence of 11.2% and affects about 743 million people (Kassebaum et al. 2014). In general, it affects about 20–50% of the

world's population (Nazir 2017) and is the most prevalent condition of the oral cavity among human population (Marushchak et al. 2017a). In Ukraine, the prevalence of periodontal disease among the population is ranging from 92 to 98%, and the most common are chronic catarrhal gingivitis and generalized periodontitis (GP) (Antonenko et al. 2016; Shevchuk 2018). There is evidence that even 15–18-year-olds, in 55–89% of cases, suffer from gingivitis or the initial stage of GP (Gasiuk 2015). Overall, the high

prevalence of inflammatory periodontal diseases, their multifactority, predisposition to progression, significant difficulties in achieving of sustainable remission, a marked increase of the number of young people with severe destructive and atrophic periodontal changes, the impact on the general condition of the body and reduced quality of life, permit GP to consider as a chronic non-communicable disease (NCD). It shares social determinants and risk factors with the major NCDs, resulting in approximately two-thirds of deaths, such as heart disease, diabetes, cancer, and chronic respiratory disease (Krynytska et al. 2017; Tonetti et al. 2017a, b; Marushchak et al. 2019a, b; Degen et al. 2020).

Among the leading factors of the GP pathogenesis, a microbial factor – bacterial colonization of the cervical surface of the teeth occurs in the form of “bacterial plaques” and induce an invasion of microorganisms into periodontal tissues (Shcherba et al. 2020). Microbial enzymes cause depolymerization of proteoglycans and glycosaminoglycans of the main substance of the periodontium and disruption of their resynthesis that results in a possible invasion of endotoxins into tissues (Mykytenko 2015). In addition, microbial aggression and inflammation contribute to the accumulation and activation of osteoclasts, which leads to degradation of the organic matrix and destruction of bone tissue (Sevishko 2006).

At the same time, the periodontopathogenic microflora is a source of endotoxins, which, in addition to the direct harmful effect on the periodontal tissue, entering the blood, stimulate the increase of pro-inflammatory cytokines production, and acute phase reactants. According to Duvina et al. (2012), the progressive destruction of bone and connective tissues in patients with periodontitis is associated with a combination of cytokines such as interleukins IL-1 β and IL-6, tumor-necrosis factor- α (TNF- α) and prostaglandin E₂, which activate osteoclastogenesis and the bone tissue resorption by osteoclasts. Under the action of pro-inflammatory cytokines, periodontal tissues are infiltrated by neutrophils and macrophages. The process of the secretory degranulation of these cells is triggered by the release of matrix metalloproteinases (MMPs) (Savitri et al. 2015; Zhang and Li 2015), which in turn causes destruction of connective tissue and subsequent destruction of alveolar bone, activating resident periodontal cells (Bhankhar et al. 2017). At the same time, there is evidence that the level of cytokines in gingival crevicular fluid and peripheral blood does not always correlate with the processes of the bone remodeling (Tsarev et al. 2013).

The GP has been proven to be pathogenetically

closely related to the somatic pathology (Albandar et al. 2018). Particularly relevant are the problems of hormonal regulation of inflammatory reactions in the periodontium in a setting of the thyroid pathology. After the Chernobyl accident in 1986, an increase in the incidence of thyroid dysfunction has been registered, mainly in Central, Northern, and Eastern Europe. In Ukraine, the number of thyroid disorders has been increased 5 times over the past 5 years (Bilous et al. 2020). Despite the large number of studies on the effect of thyroid dysfunction on the course of the GP (Zahid et al. 2011; Gupta et al. 2014; Beriashvili et al. 2016; Kothiwale and Panjwani 2016; Rahangdale and Galgali 2018; Aldulaijan et al. 2020), they are often contradictory and the molecular mechanisms of thyroid hormone action on periodontal disease have not been fully revealed.

This study was aimed to investigate the content of the inflammatory mediators in rat with only the periodontitis and the periodontitis in a setting of hyper- and hypothyroidism and to analyze the correlative linkages between the inflammatory mediators and the thyroid hormones.

Materials and methods

Animals and study design. White male 12–14 weeks old inbred rats (n=48) weighing 180–200 g were employed in the experiment. During the period of the experiment, the animals were kept under standardized conditions, with an adjustable light cycle (12/12) and unrestricted access to water and food *ad libitum* at the animal facility of I. Horbachevsky Ternopil National Medical University. The treatment of the animals and all experimental procedures were performed in compliance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (European convention for the protection of vertebrate animals used for experimental and other scientific purposes 1986). The Bioethics Commission of I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine approved the protocol of the experiment (Excerpts from Minutes No. 59, dated 23.10.2019).

The animals were randomly divided into the following groups: Group I: animals in the control group injected with an intragastric 1% starch solution (n=12); Group II: animals with a model of periodontitis (n=12). The rats in this group were two weeks injected with 40 μ l (1 mg/ml) of *E. coli* lipopolysaccharide (LPS) (Sigma-Aldrich, USA) into gingival tissues every other day (Moyseeva 2008).

Group III: rats with periodontitis in a setting of hyperthyroidism ($n=12$). The animals of this group every day received intragastric doses of L-thyroxine in a 1% starch solution at 10 $\mu\text{g/day}$ per 100 g of body weight for 21 days to create an experimental model of thyroid hyperfunction (Ratushnenko 2010). From day 8, the rats received LPS into the gingival tissue for two weeks; Group IV: included rats with periodontitis in a setting of hypothyroidism ($n=12$). The animals received every day intragastric doses of methimazole in a 1% starch solution at 1 mg/day per 100 g of body weight for 21 days to create an experimental model of thyroid hypofunction (Ratushnenko 2010). From day 8, the rats received LPS into the gingival tissue for two weeks.

On day 22 from the onset of the experiment, the rats were euthanized under deep thiopental-sodium anesthesia by cardiac puncture. Blood and periodontal samples were used for further investigations.

To prepare 10% homogenate, periodontal samples, taken immediately after euthanasia, were cooled to 1–3°C in saline, dried with filter paper, and homogenized in 0.05 M Tris-HCl buffer (pH 7.4) using a magnetic homogenizer SilentCrusher S, (Heidolph, Germany). The ratio of tissue mass to buffer volume was 1: 9. The obtained homogenate was centrifuged at 1500 $\times g$ for 30 min on a centrifuge with cooling Hermle Z 32 HK using supernatant for research (Avdeev 2013). To obtain blood serum, blood samples were allowed to clot at room temperature for 30 min and then they were centrifuged at 1200 $\times g$ for 15 min in room temperature.

Blood serum levels of free thyroxine (fT4), free triiodothyronine (fT3), and thyroid-stimulating hormone (TSH), were analyzed with ELISA kits manufactured by Vector Best (Russia). The contents of TNF- α , IL-1 β , and IL-10 in the periodontal homogenate supernatant were studied by a solid-phase enzyme-linked immunosorbent assay using RayBio reagent kits manufactured by RayBiotech (USA) according to the manufacturer's protocol on a Multiscan FC analyzer (Thermo Scientific, Finland) and expressed as pg/ml.

Statistical analysis. The experimental data were processed and analyzed using MS Office 2016 EXCEL (Microsoft Corp., USA) and STATISTICA 13 (TIBCO Software Inc., 2018). The distribution of data was analyzed in accordance with the assessment of normality by the Kolmogorov-Smirnov criterion. The obtained values had a normal distribution, so the difference between the groups was analyzed using the Student's *t*-criterion. All data were presented as mean $\pm$ standard error of mean (SEM). A probability

level (*p*-value) of less than 0.05 was considered to be statistically significant. The relationship between the studied indicators was established based on the results of the correlation analysis using the Pearson's correlation coefficient. The linear correlation coefficient (*r*) and its probability (*p*) were calculated, which was correspondingly indicated in the tables (correlation matrices). The relationship was considered very weak at a correlation coefficient $r=0.1-0.3$, weak at $r=0.3-0.5$, moderate at $r=0.5-0.7$, strong at $r=0.7-0.9$, and very strong at $r=0.9-0.99$. The direction of the relationship, direct or inverse, was also assessed. The correlation coefficient was evaluated as statistically significant at $p<0.05$.

Results

We found that three weeks of L-thyroxine administration caused hyperthyroidism, in rats, which was confirmed by a decrease in the TSH serum levels (by 2.3 times) and an increase of fT4 serum levels (by 1.8 times) (Table 1) compared to control animals. The fT3 levels underwent the smallest changes, which were increased, but only by 1.3 times ($p<0.01$). The antithyroid agent, mercazolil, was used to model the condition of hypothyroidism. The mechanism of its thyrostatic action is due to the inhibition of peroxidase activity, the enzyme involved in the formation of T4 and T3, and the inhibition of thyronine iodination and reduced T4 increment. Administration of mercazolil to rats for 3 weeks caused hypothyroidism, which was confirmed by a 2.3-fold decrease of fT4 serum levels ($p<0.02$). The levels of TSH significantly increased by 51.3% compared to controls. The least

Table 1
Changes of free thyroxine (fT4), free triiodothyronine (fT3) and thyroid-stimulating hormone (TSH) levels in the blood serum of rats injected with L-thyroxine and mercazolil

Parameter	Groups of animals		
	Control	L-thyroxine	Mercazolil
TSH [mIU/l]	0.37 $\pm$ 0.03	0.16 $\pm$ 0.02 $p_1<0.001$	0.56 $\pm$ 0.04 $p_1<0.001$ $p_2<0.001$
fT3 [pmol/l]	5.41 $\pm$ 0.17	6.92 $\pm$ 0.23 $p_1<0.01$	4.08 $\pm$ 0.16 $p_1<0.01$ $p_2<0.001$
fT4 [pmol/l]	15.07 $\pm$ 0.48	26.64 $\pm$ 1.02 $p_1<0.001$	6.65 $\pm$ 0.44 $p_1<0.001$ $p_2<0.001$

Data are presented as mean $\pm$ standard error of mean ($n=12$); p_1 – significant differences in comparison to control animals; p_2 – significant differences between experimental groups.

pronounced changes underwent again the level of fT3, which decreased by 24.6% ($p < 0.01$) compared to control animals.

TNF- α is one of the main mediators that determines the development and progression of inflammation in periodontal tissues (Sabirova 2016). It was found that the TNF- α levels increased by 2.4 times ($p < 0.001$) in the periodontal homogenate supernatant in rats with only periodontitis and by 2.7 times ($p < 0.001$) in rats with periodontitis in a setting of hypothyroidism vs. control group (Table 2). The TNF- α levels underwent maximum changes. They increased by 3.3 times ($p < 0.001$) in animals with periodontitis in a setting of hyperthyroidism. Comparing the data in animals of different experimental groups, a number of significant differences: excess by 38.5% ($p < 0.01$) in rats with periodontitis and hyperthyroidism vs. rats with only periodontitis; excess by 20.1% ($p < 0.05$) in rats with periodontitis and hyperthyroidism vs. rats with periodontitis and hypothyroidism were revealed. When comparing the obtained data in rats with only periodontitis vs. rats with periodontitis and hypothyroidism, no significant changes were found.

IL-1 β is an important mediator of an inflammatory response and is involved in the pathophysiology of periodontitis (Oh et al. 2015). The IL-1 β

levels increased by 2.0 times ($p < 0.001$) in a setting of periodontitis without concomitant pathology and by 2.4 times ($p < 0.001$) in rats with periodontitis in a setting of hypothyroidism vs. control group. This indicator was most pronounced in animals with periodontitis in a setting of hyperthyroidism. IL-1 β levels increased by 2.9 times ($p < 0.001$) compared to controls. Comparing the obtained data in animals of different experimental groups, a number of significant differences: excess by 47.9% ($p < 0.001$) in rats with periodontitis and hyperthyroidism vs. rats with only periodontitis; excess by 19.5% ($p < 0.05$) in rats with periodontitis and hypothyroidism vs. rats with only periodontitis; excess by 23.7% ($p < 0.01$) in rats with periodontitis and hyperthyroidism vs. rats with periodontitis and hypothyroidism, were revealed.

Regarding the change in the anti-inflammatory IL-10 levels, a decrease by 56.9% ($p < 0.001$) in the periodontal homogenate supernatant of animals with periodontitis without concomitant pathology and by 68.7% ($p < 0.001$) in rats with periodontitis in a setting of hypothyroidism vs. control group were found. The most pronounced decrease at IL-10 levels (by 73.8%; $p < 0.001$) in animals with periodontitis was detected in a setting of hyperthyroidism. Comparing the obtained data in animals in different experimental

Table 2

The indicators of inflammatory mediators in periodontal homogenate supernatant of rats with only periodontitis and in a setting of hyper- and hypothyroidism

Parameter	Groups of animals			
	Control	Periodontitis	Periodontitis in a setting of hyperthyroidism	Periodontitis in a setting of hypothyroidism
TNF- α (pg/ml)	1.70 $\pm$ 0.20	4.05 $\pm$ 0.37 $p_1 < 0.001$	5.61 $\pm$ 0.32 $p_1 < 0.001$ $p_2 < 0.01$	4.67 $\pm$ 0.24 $p_1 < 0.001$ $p_3 > 0.05$ $p_4 < 0.05$
IL-1 β (pg/ml)	4.55 $\pm$ 0.36	9.07 $\pm$ 0.65 $p_1 < 0.001$	13.41 $\pm$ 0.60 $p_1 < 0.001$ $p_2 < 0.001$	10.84 $\pm$ 0.29 $p_1 < 0.001$ $p_3 < 0.05$ $p_4 < 0.01$
IL-10 (pg/ml)	7.21 $\pm$ 0.30	3.11 $\pm$ 0.15 $p_1 < 0.001$	1.89 $\pm$ 0.15 $p_1 < 0.001$ $p_2 < 0.001$	2.26 $\pm$ 0.13 $p_1 < 0.001$ $p_3 < 0.002$ $p_4 > 0.05$
IL-1 β /IL-10	0.63 $\pm$ 0.04	3.01 $\pm$ 0.28 $p_1 < 0.001$	7.99 $\pm$ 1.08 $p_1 < 0.001$ $p_2 < 0.001$	5.10 $\pm$ 0.54 $p_1 < 0.001$ $p_3 < 0.002$ $p_4 < 0.01$

Data are presented as mean $\pm$ standard error of mean ($n=12$); p_1 – significant differences in comparison to control animals; p_2 – significant differences between the group of only periodontitis with the group of periodontitis in combination with hyperthyroidism; p_3 – significant differences between the group of only periodontitis with the group of periodontitis in combination with hypothyroidism; p_4 – significant differences between the group of periodontitis in combination with hyperthyroidism with the group of periodontitis in combination with hypothyroidism. Abbreviations: TNF- α – tumor necrosis factor- α ; IL-1 β – interleukin-1 β ; IL-10 – interleukin-10.

groups, a number of significant differences: excess by 64.6% ($p < 0.001$) in rats with periodontitis and hyperthyroidism vs. rats with only periodontitis; excess by 37.6% ($p < 0.002$) in rats with periodontitis and hypothyroidism vs. rats with only periodontitis were revealed. Comparing the obtained data in rats with periodontitis and hyperthyroidism vs. rats with periodontitis and hypothyroidism, no significant changes were found.

The ratio of cytokines with pro-inflammatory and anti-inflammatory activities (IL-1 β /IL-10) increased by 4.8 times in rats with periodontitis without concomitant pathology vs. control group. In rats with periodontitis in a setting of hyperthyroidism, this indicator was increased by 12.7 times vs. control group. It should be noted that the ratio of IL-1 β /IL-10 was 2.7 times ($p < 0.001$) higher in hyperthyroid rats

than in rats with periodontitis without concomitant pathology and 1.6 times ($p < 0.01$) higher in rats with periodontitis in a setting of hypothyroidism. In animals with periodontitis in a setting of hypothyroidism, the ratio of IL-1 β /IL-10 increased by 8.1 times ($p < 0.001$) in the periodontal homogenate supernatant vs. control group 1.7 times higher than the data of group with only periodontitis.

By analyzing the correlative linkages between the content of pro-inflammatory cytokines (TNF- α and IL-1 β) in the periodontal homogenate supernatant and the levels of thyroid hormones in the blood serum of hyperthyroid rats with periodontitis, the inverse relationship between TSH and both studied cytokines and the direct relationship between fT3 and IL-1 β were established (Tables 3, 4). In the hypothyroid rats with periodontitis, the content of

Table 3
Correlative linkages between TNF- α contents in periodontal homogenate supernatant and TSH, fT3 and fT4 levels in blood serum of hyper- and hypothyroid rats with periodontitis

Correlative linkages		Experimental group	Coefficient of linear correlation, r_{xy}	Fidelity of correlative linkage, p-value
TSH (mIU/l)	TNF- α (pg/ml)	Periodontitis in combination with hyperthyroidism	-0.62	<0.05
		Periodontitis in combination with hypothyroidism	0.37	>0.05
fT3 (pmol/l)	TNF- α (pg/ml)	Periodontitis in combination with hyperthyroidism	0.39	>0.05
		Periodontitis in combination with hypothyroidism	-0.59	<0.05
fT4 (pmol/l)	TNF- α (pg/ml)	Periodontitis in combination with hyperthyroidism	0.34	>0.05
		Periodontitis in combination with hypothyroidism	-0.61	<0.05

Abbreviations: TSH – thyroid-stimulating hormone; fT3 – free triiodothyronine; fT4 – free thyroxine; TNF- α – tumor necrosis factor- α .

Table 4
Correlative linkages between IL-1 β contents in periodontal homogenate supernatant and TSH, fT3 and fT4 levels in blood serum of hyper- and hypothyroid rats with periodontitis

Correlative linkages		Experimental group	Coefficient of linear correlation, r_{xy}	Fidelity of correlative linkage, p-value
TSH (mIU/l)	IL-1 β (pg/ml)	Periodontitis in combination with hyperthyroidism	-0.62	<0.01
		Periodontitis in combination with hypothyroidism	0.46	>0.05
fT3 (pmol/l)	IL-1 β (pg/ml)	Periodontitis in combination with hyperthyroidism	0.60	<0.05
		Periodontitis in combination with hypothyroidism	-0.51	>0.05
fT4 (pmol/l)	IL-1 β (pg/ml)	Periodontitis in combination with hyperthyroidism	0.43	>0.05
		Periodontitis in combination with hypothyroidism	-0.68	<0.05

Abbreviations: TSH – thyroid-stimulating hormone; fT3 – free triiodothyronine; fT4 – free thyroxine; IL-1 β – interleukin-1 β .

TNF- α inversely correlated with both fT3 and fT4. Regarding IL-1 β , the inverse correlation with only fT4 was established.

By analyzing the correlative linkages between the content of anti-inflammatory IL-10 in the periodontal homogenate supernatant and the levels of thyroid hormones in the blood serum of hyperthyroid rats, a strong direct correlation with TSH, a moderate inverse correlation with fT3, and a strong inverse correlation with fT4 were established (Table 5). In hypothyroid rats, a moderate inverse correlation with TSH, a moderate direct correlation with fT3, and a strong direct correlation with fT4 were found.

Discussion

Cytokines are integral components of the complex intercellular communication that are necessary for production and control of an immune response. Some of them are expressed as membrane-bound proteins, while others are secreted in the form of soluble molecules that are aimed at high-affinity receptors on the surfaces of neighboring cells. Each cytokine signal, by binding either to a unique or a shared receptor, causes an intracellular signalling cascade that can lead to up- or down-regulation of transcription factors that regulate the expression of various other genes. This can lead to the formation of other biologically active molecules, including other cytokines, a change in the number of surface receptors, or a feedback inhibition loop leading to self-regulation (Ganesh et al. 2011; Marushchak et al. 2017b).

It has been shown that the master pro-inflammatory cytokine, TNF- α , modulates many signalling

pathways, with wide range of downstream effects (Marushchak et al. 2017c; Holbrook et al. 2019). TNF- α stimulates the production of other pro-inflammatory cytokines, increases the expression of intercellular and vascular adhesion molecules that are involved in the migration of lymphocytes into the pathological focus, proliferation of fibroblasts and synoviocytes, stimulates the formation of MMPs and suppresses the synthesis of their inhibitors, activates osteoclasts (Sabirova 2016). *In vitro* studies of the effects of TNF- α on human osteoblasts culture have shown that it stimulates proliferation in low doses and significantly inhibits proliferation when administered in high doses or over a long period of time (Deo and Bhongade 2010). In addition, TNF- α inhibits the differentiation of osteoblast progenitor cells. This effect is due to its inhibition of runt related transcription factor 2 (RUNX2) (Gilbert et al. 2002).

The results of our study showed that the content of TNF- α in the periodontal homogenate supernatant in rats with only periodontitis significantly increased vs. control group. Schmidt (2009), in his study on cytokines in the gingival crevicular fluid (GCF) of patients with GP, has also found a marked increase of TNF- α content, which was more than 7.0 times higher than the level of healthy individuals. Similar results have been obtained by Al-Ghurabi and co-authors (2013), who have found an increase in serum TNF- α levels in patients with chronic periodontitis.

The synthesis of TNF- α in gum tissue can activate another cytokine, IL-1 (Pan et al. 2019), which is a system of three cytokines: IL-1 α , IL-1 β , IL-1Ra and two receptors R₁ and R₂ (Alexandrov 2014). It has been shown that the progression of GP in peri-

Table 5
Correlative linkages between IL-10 contents in periodontal homogenate supernatant and TSH, fT3 and fT4 levels in blood serum of hyper- and hypothyroid rats with periodontitis

Correlative linkages		Experimental group	Coefficient of linear correlation, r_{xy}	Fidelity of correlative linkage, p-value
TSH (mIU/l)	IL-10 (pg/ml)	Periodontitis in combination with hyperthyroidism	0.71	<0.01
		Periodontitis in combination with hypothyroidism	-0.60	<0.05
fT3 (pmol/l)	IL-10 (pg/ml)	Periodontitis in combination with hyperthyroidism	-0.65	<0.05
		Periodontitis in combination with hypothyroidism	0.58	<0.05
fT4 (pmol/l)	IL-10 (pg/ml)	Periodontitis in combination with hyperthyroidism	-0.76	<0.01
		Periodontitis in combination with hypothyroidism	0.71	<0.01

Abbreviations: TSH – thyroid-stimulating hormone; fT3 – free triiodothyronine; fT4 – free thyroxine; IL-10 – interleukin-10.

odontal tissues and GCF, increases the content of IL-1 (Zyablov *et al.* 2011; Batra *et al.* 2019). Its increased secretion probably disrupts the mechanisms of inverse relationship that limit inflammation, leading to the formation of massive pockets in the gingival sulcus and degradation of periodontal tissue (Tsarev *et al.* 2013). Acting on endothelial cells, IL-1 increases the synthesis of adhesion molecules for neutrophils and monocytes, which ensures their mobilization in periodontal tissues and in fibroblasts enhances the production of collagenase, which destroys gingival attachments and stimulates osteoclasts in the bone tissue of the jaws. One report has suggested that the role of IL-1 in osteoclastic bone resorption is partially mediated by induction of prostaglandin E2 (PGE2) release (Richards and Rutherford 1988).

The dominant form of IL-1 is multifunctional IL-1 β , which is mainly produced by which are macrophages and monocytes, as well as lymphocytes, fibroblasts and endotheliocytes (Alexandrov 2014). Several studies have reported a correlation between the inflammatory status of periodontal disease and IL-1 β levels in gingival tissues and GCF (Al-Shammari *et al.* 2001; Engebretson *et al.* 2002; Faizuddin *et al.* 2003). Figueredo *et al.* (1999) have reported that the IL-1 β levels in GCF were induced in samples from patients with periodontitis, regardless of the severity of disease at the sampling site. The study of Oh *et al.* (2015) have suggested that IL-1 β levels in serum samples of thirteen nonsmoking patients, who suffer from chronic periodontitis, depended on the severity of inflammation.

In our studies, we noted a significant increase of IL-1 β levels in the periodontal homogenate supernatant, although less pronounced than TNF- α . Our results are consistent with the data of Al-Ghurabi and co-authors (2013), who have found an increase in the serum IL-1 β levels in patients with GP. In contrast, other researchers have found no significant changes of IL-1 β and TNF- α levels in patients with chronic periodontitis and healthy individuals (Ikezawa-Suzuki *et al.* 2008; Elkhoul 2011).

The anti-inflammatory interleukin IL-10 is the key regulator of the immune response. Publications regarding the IL-10 presence in periodontal tissues are not numerous (Dutzan *et al.* 2009; Pan *et al.* 2019). We found that its levels significantly decreased by 56.9% in the periodontal homogenate supernatant of rats with only periodontitis vs. control group. The obtained data are consistent with the results of Schmidt (2009), who has found a 2-fold decrease of IL-10 levels in the GCF in a setting of GP vs. control group.

In case of LPS-induced inflammation in a setting of thyroid dysfunction, we found a more pronounced hyperproduction of pro-inflammatory cytokines (TNF- α , IL-1 β) in case of a more pronounced decrease of anti-inflammatory cytokine IL-10 relative to periodontitis without concomitant pathology. Comparing the changes of the cytokine profile in the periodontal homogenate supernatant of hyper- and hypothyroid rats with periodontitis, a higher intensity of changes was found in a setting of hyperthyroidism. This suggests that thyroid dysfunction, especially its hyperfunction, may play an important role in pro-inflammatory response in periodontitis. In addition, an imbalance between pro- and anti-inflammatory cytokines, in a setting of thyroid dysfunction, may be involved in the initiation and advancement of periodontitis and indicates a stronger systemic proinflammatory state in disease.

Thyroid hormones, notably T3 and T4, modulate specific immune responses that include cell-mediated immunity, activity of natural killer cell, and T and B lymphocytes proliferation. The effects of thyroid hormones are mediated by specific thyroid hormone receptors that are located in nuclei or can transfer from the cytoplasm into the cell nucleus to regulate gene expression. Although both T3 and T4 enter the cells, only T3 is a ligand for the thyroid hormone receptors (De Vito *et al.* 2011). There is evidence that T3 stimulates the production of IL-6, IL-8, enhances the effects of IL-1, IL-6, and increases the proliferation, differentiation and apoptosis of osteoblasts (Tuchendler and Bolanowski 2014). During bone resorption, T3 increases the expression of important osteoclast differentiation factors, such as IL-6 and PGE-2, which is a potent, multifunctional regulator of bone metabolism (Kalajzic *et al.* 2014). In addition, T3 has been shown to increase the expression of NF- κ B ligand (RANKL) mRNA in osteoblasts, which activates RANK that is present in osteoclast precursors (Cardoso *et al.* 2014). Thus, NF- κ B-dependent processes associated with the receptor activator of NF- κ B (RANK), its ligand (RANKL), and a decoy receptor osteoprotegerin, are important regulators of osteoclast resorption activity (Ambili and Janam 2017) affected by thyroid dysfunction.

Grover and Luthra (2013) have noted that excessive production of cytokines in a setting of thyroid dysfunction disrupts tissue repair and activates bone resorption. It has been proved that the most destructive effect on periodontal tissues is shown by TNF- α , due to the activation of osteoclastogenesis. Monea and co-authors (2014) have also noted that IL-6 and TNF- α , which are generated in a setting of thyroid

dysfunction, play an important role in the initiation processes and enhancement of the inflammatory cascade in periodontal tissues. Kanatani and co-authors (2004) have pointed to the negative impact of thyroid dysfunction on IL-6 and TNF- α cytokines that are responsible for osteoclast differentiation and act independently of the RANKL-mediated mechanism. Maklakova and co-authors (2019) have found a significant increase of IL-10 content in all patients with diffuse toxic goiter, especially at the onset of the disease, which correlated with the severity of thyrotoxicosis. A similar result has been obtained by Jiskra and co-authors (2009) who have found an increase in IL-10 and TNF- α levels in patients suffering from diffuse toxic goiter compared with autoimmune thyroiditis patients and healthy individuals. In addition, these researchers have found significant interactions between IL-2 levels in patients with diffuse toxic goiter and thyroid hormone level a direct correlation with fT3 and an inverse correlation with TSH. Similar correlations were determined between the content of IL-4 and the level of thyroid hormones, the level of IL-4 positively correlated with the contents of fT3 and fT4, or negatively with TSH (Saprina et al. 2012). Makay and co-authors (2009) have found an increase of TNF- α content in the blood serum of rats that were in a state of drug-induced thyrotoxicosis. In the study of Serebryakova (2008), increased serum TNF- α levels in complicated forms of thyrotoxicosis has been found, what is consistent with the data of Kravets and co-authors (2010) on the growth of this cytokine levels in the blood serum of patients with diffuse toxic goiter. However, the researchers have suggested that the increase of TNF- α content depends rather more on the activity of the autoimmune process directed at the target organ, than on the level of thyroid hormones. It is believed that TNF- α is directly involved in the production of autoantibodies. It should be noted that the authors

of some studies indicate a decrease of the production of TNF- α at diffuse toxic goiter or production close to normal (Saprina et al. 2012). At the same time, Gryaznova and Khamnueva (2017) have noted a pronounced hypercytokinemia in patients with a hypothyroidism in a setting of autoimmune thyroiditis. Treatment for 4–6 months had virtually no effect on cytokine levels, despite the normalization of TSH and fT4 levels as well as the relief of clinical manifestations. Popova and co-authors (2013) have found that in patients with subclinical hypothyroidism, the levels of IL-8 and IL-10 are increased, and when the level of TSH that is characteristic of euthyroidism reached the levels of these cytokines also normalized.

Conclusions

It was shown that experimental LPS-induced periodontitis is accompanied by hyperproduction of pro-inflammatory cytokines (TNF- α and IL-1 β) and reduction of anti-inflammatory cytokine (IL-10), whereas TNF- α undergoes maximum changes. Thyroid dysfunction exacerbates cytokine imbalance and severity of inflammation in experimental LPS-induced periodontitis, especially pronounced at hyperthyroidism, as evidenced by the predominance of TNF- α and IL-1 β contents in the periodontal homogenate supernatant by 38.5 % ($p < 0.01$) and 75.6% ($p < 0.001$), respectively, of hyperthyroid over the euthyroid and by 20.1% ($p < 0.05$) and 24.1% ($p < 0.05$), respectively, over the hypothyroid rats. This suggests that thyroid dysfunction, especially its hyperfunction, may play an important role in pro-inflammatory response in periodontitis. Hyperproduction of inflammatory mediators in thyroid dysfunction can cause a noticeable damage in the whole apparatus of the periodontium thereby causing progression of periodontitis.

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