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Correlative link ages between indices of bone metabolism and thyroid hormones in rats with periodontitis

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Abstract

Introduction: It has been established that changes in the bone tissue of the jaw are present in all cases where there are at least small pathological inflammatory changes in the mucous membrane of the oral cavity. This suggests a significantly greater pathogenetic relationship between inflammatory changes in the mucosa and changes in the bone part of the periodontal disease. Despite a large number of studies, the molecular mechanisms of the influence of thyroid hormones on the bone metabolism have not been completely studied.

The aim of study: to clarify mechanisms of the periodontitis development in rats with thyroid dysfunction based on a comparative analysis of the correlations between the bone metabolism indices and the concentration of thyroid stimulating hormone, free thyroxine and free triiodothyronine.

Material and methods: Experimental studies were conducted on male, nonliner, white rats of around 4 months of age. The experimental animals were divided into the following groups: I – control animals; II – animals with periodontitis; III – animals with periodontitis combined with hyperthyroidism; IV – animals with periodontitis combined with hypothyroidism. Total calcium, ionized calcium, phosphorus, osteocalcin concentration and activity of phosphatases were measured. Correlation analysis was performed between all the studied indices. Coefficient of linear correlation (r) and its fidelity (p) was calculated that was accordingly denoted in the tables (correlation matrices). The correlation coefficient was significant at $p<0.05$.

Results: The conducted correlative analysis shows that there are different interconnections between the indices of calcium-phosphorus metabolism, bone formation and bone resorption with free triiodothyronine, free thyroxine and thyroid stimulating hormone, in case of the experimental periodontitis combined with thyroid dysfunction. In animals with modelled periodontitis combined with hyperthyroidism, more correlative interactions with triiodothyronine, thyroxine and thyroid stimulating hormone were established compared to the rats of hypothyroid group.

Conclusions: Consequently, the thyroid dysfunction significantly affects the parameters of bone metabolism in case of an experimental periodontitis, which requires further research.

Key words: periodontitis, thyroid hormones, bone metabolism, correlations.

Introduction

Specific types of connective tissue (bone tissue, dentin, cement and dental enamel) are characterized by a high content of the mineral component, the main part of which are calcium salts Bone tissue of the jaw has a characteristic organization of structural elements (osteons and trabeculae). Despite the high degree of mineralization, there is a constant renewal of parts substances in the bone tissue, permanent destruction and creation, adaptive rearrangements to constantly changing conditions of functioning [8].

The main "actors" of this process are circulating in the extracellular fluid levels of calcium, phosphorus, as well as the hormones that regulate their metabolism. Calcium performs only one function in the intercellular matrix - structural. At the same time, calcium of the mineral component of the intercellular matrix is a depot for intracellular calcium, where it acts as a mediator in mechanisms of intracellular transfer of signals entering the cell. Phosphorus, to a certain extent, is involved in almost all metabolic processes. Calcium and phosphorus form the basis of solid bone matter. Phosphorus is represented by a slightly soluble calcium phosphate (2/3) and soluble compounds (1/3) in bones. The vast majority of the phosphorus is inside the cells, 1% is in the extracellular fluid. Mineral components of bone tissue are in a state of chemical equilibrium with calcium and phosphate ions of serum [4, 10].

According to the literature, it has been established that changes in the bone tissue of the jaw are present in all cases where there are at least small pathological inflammatory changes in the mucous membrane of the oral cavity. This suggests a significantly greater pathogenetic relationship between inflammatory changes in the mucosa and changes in the bone part of the periodontal disease. It is likely that these changes occur simultaneously [8].

Masalova N.N. and Zakharenko R.V. argue that dysfunction of the thyroid gland is accompanied by changes in phosphorous-calcium and bone metabolism. Hyperthyroidism causes an increase in bone turnover due to rising the number of osteoclasts and resorption surfaces, as well as a violation of the ratio of resorption and bone formation spaces, i.e. an increase in both the parameters of bone formation and resorption. Enhanced bone remodelling during of hyperthyroidism is caused by an increasing in the activity of osteoclasts and osteoblasts, which leads to a simultaneous increasing in the rate of its resorption and formation. In this case, the processes of resorption dominate bone formation, develops a negative calcium balance, which leads to a decreasing in mineral density of bone tissue [5].

In patients with untreated hypothyroidism, signs of decreased bone formation and bone resorption have been observed. They tend to reduce the excretion of calcium from the urine, as well as an increase in the level of parathyroid hormone (PTH) and vitamin D_3. The levels of calcium and phosphorus in the blood and urine in most cases remain without significant changes. According

to some authors [3, 11], hypothyroidism is characterized by resistance to the effects of PTH, with the level of intact PTH most often remaining in the normal level, but there are a number of reports indicating an increase in the level of PTH and vitamin D3, which to some extent supports the concept of resistance to PTH [5].

Despite a large number of studies, the molecular mechanisms of the influence of thyroid hormones on the bone metabolism have not been completely studied. The effect of thyroxine (T4) and triiodothyronine (T3) on the metabolism of periodontal tissue in the condition of generalized periodontitis shows a special interest.

The aim of the study: to clarify mechanisms of the periodontitis development in rats with thyroid dysfunction based on a comparative analysis of the correlations between the bone metabolism indices and the concentration of thyroid stimulating hormone, free thyroxine and free triiodothyronine.

Material and methods: Experimental studies were conducted on male, nonliner, white rats of around 4 months of age, that were housed at 25±3 °C and humidity of 55±2 %, under a constant 12 h light and dark cycle. Water was available ad libitum. The experimental animals were divided into the following groups: I – control animals administered intragastrically 1% solution of starch (n=12); II – animals with periodontitis (The rats of this group were injected into gum tissue with lipopolysaccharide (LPS) E. Coli («Sigma-Aldrich», США)) during 2 weeks (n=12) [6]; III – animals with periodontitis combined with hyperthyroidism. To model the experimental hyperfunction of the thyroid gland, animals received intragastrically L-thyroxine daily in 1% starch solution at a rate of 10 μg/day per 100 g of body mass for 21 days (n=12) [9]. Starting from the eighth day of experiment, rats were injected into gum tissue with LPS E. Coli during 2 weeks; IV – animals with periodontitis combined with hypothyroidism. To model the experimental hypofunction of the thyroid gland, animals received intragastrically Merkazolil daily in 1% starch solution at a rate of 1 mg/day per 100 g of body mass for 21 days (n=12) [9]. Starting from the eighth day of experiment, rats were injected into gum tissue with LPS E. Coli during 2 weeks.

Animal euthanasia was carried out on the 22^{d} day of the experiment by cardiac puncture under deep anaesthesia, in accordance with the requirements of the European convention for the protection of vertebrate animals used for experimental and other scientific purposes [15].

For investigation, blood serum and homogenate of periodontal tissue were used, which was made on Tris/ HCl / Buffer (pH 8.0) at a rate of 100 mg of tissue per ml [1]. To confirm the conditions of hyperthyroidism and hypothyroidism the concentration of serum free thyroxine (fT4), free triiodothyronine (fT3) and thyroid stimulating hormone (TSH) were assayed by Enzyme Linked Immunosorbent Assay (ELISA) kit Vector-Best (Russia).

Total calcium and phosphorus concentration were measured by semi-automated biochemical analyzer Humalyzer 2000 (Human, Germany). Serum ionized calcium concentration was measured by ion-selective method using analyzer of electrolytes AEK-01 (Kvertimed, Ukraine). Bone metabolism was determined by the activity of phosphatases: alkaline (ALP) as a marker for the functioning of osteoblasts and acid (AP) as a marker of the intensification of osteoclasts' activity. In addition, the concentration of osteocalcin as a biochemical marker of the bone tissue formation and the rate of "bone turnover" was measured by ELISA kit «DRG» (USA).

Statistical analysis. The results were analysed using Statistica 6.1 software and presented as mean with standard error of mean. The differences between all groups were determined using one-way ANOVA, followed by post hoc Least Significant Difference test, with p. Correlation analysis was performed between all the studied indices. Coefficient of linear correlation (r) and its fidelity (p) was calculated that was accordingly denoted in the tables (correlation matrices). If the index r=0 the linkage was considered as absent, in the range 0-0,3 – the linkage was considered as weak correlation, interval of index 0.3-0.7 described linkage as medium strength and interval 0.7-1.0 pointed to strong correlation interaction. The correlation coefficient was significant at $p<0.05$.

Results and Discussion. The correlative analysis carried out between the serum concentration of total calcium and thyroid hormones in animals with periodontitis combined with hyperthyroidism (table 1) revealed a strong direct correlative linkage with fT_3 ($r=0,76$; $p<0,01$), and with fT_4 ($r=0,73$; $p<0,01$). Concerning the relationship between concentration of ionized calcium and thyroid hormones, we have found not only direct correlations with fT_3 ($r=0,63$; $p<0,05$) and fT_4, ($r=0,77$; $p<0,01$), but also the negative correlative linkage with TSH ($r=-0,67$; $p<0,05$). A strong direct correlative linkage between the concentration of total calcium in the homogenate of periodontal tissue in this group of animals and fT_3 ($r=0,83$; $p<0,01$) (table 2) has been found. Concerning the the relationship between the concentration of inorganic phosphorus and thyroid hormones in animals with periodontitis combined with hyperthyroidism, a strong direct correlative linkage was established with fT_3 in blood serum ($r=0,78$; $p<0,01$), and in the homogenate of periodontal tissue ($r=0,75$; $p<0,01$).

In animals with periodontitis combined with hypothyroidism, no reliable correlative linkages between the concentration of total calcium and thyroid hormones were found, but a strong direct correlation between the concentration of ionized calcium and fT_3 ($r=0,83$; $p<0,01$) was established. Concerning the the relationship between the concentration of inorganic phosphorus and thyroid hormones in animals with periodontitis combined with hypothyroidism, negative linkage of medium strength with TSH ($r=-0,61$; $p<0,05$) and direct correlative linkage of medium strength with fT_4 ($r=0,64$; $p<0,05$) were established. In the homogenate of the periodontal tissue of this

group of animals negative linkage of medium strength with TSH (r=-0,70; p<0,05) and direct correlative linkage of medium strength with fT_3 (r=0,59; p<0,05) have been found.

Table 1.

Correlative linkages between serum indices of calcium-phosphorus metabolism and thyroid hormones

Correlative linkages		Experimental group	Coefficient of linear correlation, r_{xy}	Fidelity of correlative linkage, p
TSH, mIU/l	Total calcium, mmol/l	Periodontitis combined with hyperthyroidism	-0,52	>0,05
		Periodontitis combined with hypothyroidism	0,27	>0,05
fT_3, pmol/l	Total calcium, mmol/l	Periodontitis combined with hyperthyroidism	0,76	<0,01
		Periodontitis combined with hypothyroidism	-0,09	>0,05
fT_4, pmol/l	Total calcium, mmol/l	Periodontitis combined with hyperthyroidism	0,73	<0,01
		Periodontitis combined with hypothyroidism	-0,37	>0,05
TSH, mIU/l	Ionized calcium, mmol/l	Periodontitis combined with hyperthyroidism	-0,67	<0,05
		Periodontitis combined with hypothyroidism	-0,38	>0,05
fT_3, pmol/l	Ionized calcium, mmol/l	Periodontitis combined with hyperthyroidism	0,63	<0,05
		Periodontitis combined with hypothyroidism	0,83	<0,01
fT_4, pmol/l	Ionized calcium, mmol/l	Periodontitis combined with hyperthyroidism	0,77	<0,01
		Periodontitis combined with hypothyroidism	0,46	>0,05
TSH, mIU/l	Phosphorus, mmol/l	Periodontitis combined with hyperthyroidism	-0,51	>0,05
		Periodontitis combined with hypothyroidism	-0,61	<0,05
fT_3, pmol/l	Phosphorus, mmol/l	Periodontitis combined with hyperthyroidism	0,78	<0,01
		Periodontitis combined with hypothyroidism	0,45	>0,05
fT_4, pmol/l	Phosphorus, mmol/l	Periodontitis combined with hyperthyroidism	0,53	>0,05
		Periodontitis combined with hypothyroidism	0,64	<0,05

The direct correlation of medium strength between the level of fT3 and the concentration of ionized calcium in blood serum of hypothyroid animals was established in the study of N.N. Pobigun [7]. A similar trend was observed by C. Schwarz and co-authors [17]: a significant direct correlation between calcium and fT3 and negative correlative linkage between these ions and the TSH in blood serum of patients with hypothyroidism.

Table 2.

Correlative linkages between periodontal tissue indices of calcium-phosphorus metabolism and thyroid hormones

Correlative linkages		Experimental group	Coefficient of linear correlation, r_{xy}	Fidelity of correlative linkage, p
TSH, mIU/l	Total calcium, mmol/kg	Periodontitis combined with hyperthyroidism	0,26	>0,05
		Periodontitis combined with hypothyroidism	-0,67	<0,05
fT_3, pmol/l	Total calcium, mmol/kg	Periodontitis combined with hyperthyroidism	0,83	<0,01
		Periodontitis combined with hypothyroidism	0,44	>0,05
fT_4, pmol/l	Total calcium, mmol/kg	Periodontitis combined with hyperthyroidism	0,43	>0,05
		Periodontitis combined with hypothyroidism	0,44	>0,05
TSH, mIU/l	Phosphorus, mmol/kg	Periodontitis combined with hyperthyroidism	0,19	>0,05
		Periodontitis combined with hypothyroidism	-0,70	<0,05
fT_3, pmol/l	Phosphorus, mmol/kg	Periodontitis combined with hyperthyroidism	0,75	<0,01
		Periodontitis combined with hypothyroidism	0,59	<0,05
fT_4, pmol/l	Phosphorus, mmol/kg	Periodontitis combined with hyperthyroidism	0,47	>0,05
		Periodontitis combined with hypothyroidism	0,30	>0,05

There are data that triiodothyronine is involved in the regulation of chondrogenesis and bone mineralization. It stimulates IL-6 and IL-8, enhances the effects of IL-1 and IL-6, enhances the synthesis of osteocalcin, type I collagen, increases proliferation, differentiation and apoptosis of osteoblasts [18]. During the formation of the bone, T3 stimulates proliferation, differentiation and apoptosis of osteoblasts and increases the expression of osteocalcin, type 1 collagen, alkaline phosphatase, metalproteins, IGF-1 and its receptor (IGF-1R). Subsequently, during bone resorption, T3 increases the expression of important osteoclasts differentiation factors such as interleukin-6 and prostaglandin E2. Prostaglandins, especially PGE2, are powerful multifunctional bone metabolism regulators. PGE2 causes morphological changes in osteoblasts and osteoklasts due to increased intracellular levels of cyclic adenosine monophosphate [14]. In addition, T3 acts synergistically with osteoclastogenic hormones such as PTH and vitamin D. It has also been shown that T3 increases expression of the mRNA of the receptor activator of the nuclear factor-κβ (RANKL) in the osteoblasts, which activates RANK present in the precursors of osteoclasts [12]. T4 can also

affect bone remodeling, accelerating osteoclastic activity by stimulating prostaglandin secretion [13].

The correlative analysis carried out between the serum concentration of osteocalcin and thyroid hormones in animals with periodontitis combined with hyperthyroidism (table 3) revealed a strong direct correlative linkage with fT_3 ($r=0,71$; $p<0,01$). A direct correlative linkage of medium strength between the concentration of osteocalcin and TSH ($r=0,62$; $p<0,05$), strong direct correlative linkage with fT_3 ($r=0,89$; $p<0,01$) and direct correlative linkage of medium strength with fT_4 ($r=0,59$; $p<0,05$) in the homogenate of periodontal tissue in this group of animals (table 4) have been found. Concerning the relationship between the concentration of osteocalcin and thyroid hormones in animals with periodontitis combined with hypothyroidism, we have not found any reliable correlations.

Conducted correlative analysis between the activity of ALP in blood serum and thyroid hormones in animals with periodontitis combined with thyroid dysfunction (table 3) did not reveal any reliable relationships. In the homogenate of periodontal tissue of hyperthyroid animals direct correlative linkage of medium strength between the ALP activity and fT_3 ($r=0,58$; $p<0,05$) was established. In animals with periodontitis combined with hypothyroidism strong direct correlative linkage between the ALP activity and TSH ($r=0,80$; $p<0,01$) та negative correlative linkage of medium strength with fT_4 ($r=-0,70$; $p<0,05$) were established.

Table 3.

Correlative linkages between serum indices of bone metabolism and thyroid hormones

Correlative linkages		Experimental group	Coefficient of linear correlation, r_{xy}	Fidelity of correlative linkage, p
TSH, mIU/l	Osteocalcin, ng/ml	Periodontitis combined with hyperthyroidism	0,52	>0,05
		Periodontitis combined with hypothyroidism	-0,21	>0,05
fT_3, pmol/l	Osteocalcin, ng/ml	Periodontitis combined with hyperthyroidism	0,71	<0,01
		Periodontitis combined with hypothyroidism	0,55	>0,05
fT_4, pmol/l	Osteocalcin, ng/ml	Periodontitis combined with hyperthyroidism	0,32	>0,05
		Periodontitis combined with hypothyroidism	0,27	>0,05
TSH, mIU/l	ALP, mkat/l	Periodontitis combined with hyperthyroidism	0,32	>0,05
		Periodontitis combined with hypothyroidism	-0,19	>0,05
fT_3, pmol/l	ALP, mkat/l	Periodontitis combined with hyperthyroidism	0,13	>0,05
		Periodontitis combined with hypothyroidism	-0,28	>0,05
fT_4, pmol/l	ALP, mkat/l	Periodontitis combined with hyperthyroidism	0,34	>0,05
		Periodontitis combined with hypothyroidism	0,06	>0,05
TSH, mIU/l	AP, mkat/l	Periodontitis combined with hyperthyroidism	-0,42	>0,05
		Periodontitis combined with hypothyroidism	0,37	>0,05
fT_3, pmol/l	AP, mkat/l	Periodontitis combined with hyperthyroidism	0,67	<0,05
		Periodontitis combined with hypothyroidism	-0,29	>0,05
fT_4, pmol/l	AP, mkat/l	Periodontitis combined with hyperthyroidism	0,45	>0,05
		Periodontitis combined with hypothyroidism	-0,49	>0,05

Table 4.

Correlative linkages between periodontal tissue indices of bone metabolism and thyroid hormones

Correlative linkages		Experimental group	Coefficient of linear correlation, r_{xy}	Fidelity of correlative linkage, p
TSH, mIU/l	Osteocalcin, ng/mg	Periodontitis combined with hyperthyroidism	0,62	<0,05
		Periodontitis combined with hypothyroidism	0,05	>0,05
fT_3, pmol/l	Osteocalcin, ng/mg	Periodontitis combined with hyperthyroidism	0,89	<0,01
		Periodontitis combined with hypothyroidism	-0,03	>0,05
fT_4, pmol/l	Osteocalcin, ng/mg	Periodontitis combined with hyperthyroidism	0,59	<0,05
		Periodontitis combined with hypothyroidism	-0,24	>0,05
TSH, mIU/l	ALP, mkat/kg	Periodontitis combined with hyperthyroidism	-0,46	>0,05
		Periodontitis combined with hypothyroidism	0,80	<0,01
fT_3, pmol/l	ALP, mkat/kg	Periodontitis combined with hyperthyroidism	0,58	<0,05
		Periodontitis combined with hypothyroidism	-0,28	>0,05
fT_4, pmol/l	ALP, mkat/kg	Periodontitis combined with hyperthyroidism	0,28	>0,05
		Periodontitis combined with hypothyroidism	-0,70	<0,05
TSH, mIU/l	AP, mkat/kg	Periodontitis combined with hyperthyroidism	-0,87	<0,01
		Periodontitis combined with hypothyroidism	-0,53	<0,05
fT_3, pmol/l	AP, mkat/kg	Periodontitis combined with hyperthyroidism	0,86	<0,01
		Periodontitis combined with hypothyroidism	0,18	>0,05
fT_4, pmol/l	AP, mkat/kg	Periodontitis combined with hyperthyroidism	0,64	<0,05
		Periodontitis combined with hypothyroidism	0,78	<0,01

Concerning the relationship between the activity of serum acid phosphatase and thyroid hormones in animals with periodontitis combined with hyperthyroidism direct correlative linkage of medium strength ($r=0,67$; $p<0,05$) with fT_3 was established. In hypothyroid animals, no reliable correlations were found. In the homogenate of the periodontal tissue of hyperthyroid animals negative strong correlative linkage between the AP activity and TSH ($r=-0,87$; $p<0,01$), strong direct correlation with fT_3 ($r=0,86$; $p<0,01$), direct linkage of medium strength with fT_4 ($r=0,64$; $p<0,05$) were established. In the homogenate of the periodontal tissue of hypothyroid rats strong direct correlation between AP activity and TSH ($r=0,78$; $p<0,01$) was established.

Since TSH receptor expression has been demonstrated in osteoblasts and osteoclasts, this suggests that TSH can have a direct effect on these cells [16]. Some authors state that TSH plays an important role in bone metabolism, which is independent of the action of thyroid hormones [12].

Other scientific evidence suggests that TSH is seen as a negative regulator of bone turnover. Its direct action on bone marrow cells results in increased bone remodelling and osteoporosis [19].

Conclusions:

1. Thus, the conducted correlative analysis shows that there are different interconnections between the indices of calcium-phosphorus metabolism, bone formation and bone resorption with free triiodothyronine, free thyroxine and thyroid stimulating hormone, in case of the experimental periodontitis combined with thyroid dysfunction.

2. In animals with modelled periodontitis combined with hyperthyroidism, more correlative interactions with triiodothyronine, thyroxine and thyroid stimulating hormone were established compared to the rats of hypothyroid group.

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